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NERVE TUMORS OF THE FEMALE GENITALS AND PELVIS

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MINNEAPOLIS

Relatively few nerve tumors of the female generative organs have been described. The nerve tumors of the female generative tract arise from the sympathetic nervous system. The normal development of this system is described by Streeter and the general and the special pathology of nerve tumors in Penfield's handbook and in Ewing's "Neoplastic Diseases." In the recent literature there is a lack of agreement on many fundamental issues in this field, and this has been well discussed by Boeke. Schwann (1839) initiated the concept that the neurofibril is one cell from its central to its peripheral end. This same so-called neuron theory was presented by His (1889). Schwann, however, assumed that the neurofibril was fused at many points. This concept has been resurrected by Held and others. Boeke also discussed the concept of the free expansion of the single nerve fibril, and he concluded that there is no reason to renounce the supposition of local origin and differentiation.

The ectodermal origin of all parts of the nervous system is accepted by most investigators, although the terminology applied to early stages of development is still confused. One must remember, however, that the details of the early embryonal development are not yet accurately known. The development of the caudal part of the medulla, which is of major interest in relation to pelvic nerve tumors, is different from that of other parts of the nerve system. In an embryo of eleven primary segments, Grosser found no ganglion crest in the caudal segments, although the medulla was separated from the skin and had formed a canal. The material for the spinal ganglions can therefore arise only from the closed spinal tube in this region.

The embryonic nervous system arises from the ectodermal medullary groove. His (1889) thought that the "neuroblasts" and the "spongioblasts" arose from dissimilar layers of the medulla and that the "neuroblasts" formed only ganglion cells with nerve fibrils. Schaper stated that the "neuroblasts" of His differentiate to nerve cells and glia cells and may do this after having reached the ganglions. A. Kohn called them "embryonal neurocytes," but unfortunately he also used the name "neurocytes" for later stages. This led von Mihálik to a misunderstanding of Kohn's excellent work (1902 and 1907) dealing with the development of the sympathetic nervous system.

The ectodermal origin of the sympathetic nervous system (Balfour, 1877) is generally accepted. The question as to what part of the medulla itself or which spinal nerves and ganglions give rise to the sympathetic system is important, but the pathologist is more concerned in knowing what potentialities the cells have at certain times and in special parts of the nervous system and which of those potentialities may be realized in tumors.

The embryonic sympathetic cells are understood to migrate with the spinal nerves or ganglions or a part of them directly from their original place in the

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medulla to the para-aortic position of the sympathetic trunk. Objection has been raised to this theory of His Jr. by A. Kohn (1902, 1907), Held and others and supported by Ramon y Cajal (1908), Streeter, Kuntz and recently von Mihálek. Von Mihálek confirmed the finding of A. Kohn (1903 and 1907). He claimed to find proof of the theory of migrating cells and stated that the peripheral cells near the aorta are the first that develop intracellular neurofibrillar substance. Even if this be true, it does not prove that these cells are older as von Mihálik stated. It is likely that the local conditions nearer the aorta favor earlier development of the cells.

The migration theory owes its origin to unsatisfactory methods of examination. Reconstruction of section series has not been satisfactorily done. It is not necessary to refer to the many embryologic details in this field but it may be said that the migration theory, even if it could be proved, would not influence the point of view of the pathologist in trying to interpret the tumors of these tissues. Both displacement and misplacement of cells may occur during development. Neither is necessarily followed by tumor proliferation. Many nerve tumors prove malformation in cell development. This is not brought about by misplacement but is associated with disturbances in cell differentiation, which is incomplete, defective or faulty.

High hope was held for the experimental method in studying the causes of these disturbances in differentiation. A variety of results were obtained, and it is now recognized that they varied with the degree of completeness of removal of certain parts (Müller and Ingvar). Boeke pointed out that it is dangerous to draw conclusions from experimentally produced defects in development as to the histogenesis of the elements which then fail to appear. In normal development some elements induce the formation of others. The removal of the inductor can prevent the development of the other structure.

It can be concluded only that the available knowledge as to the development of the nervous system is not yet sufficiently complete to allow convincing conclusions to be drawn as to the origin of special nerve tumors.

The origin and the identity of the Schwann sheath are of importance to the pathologist. This sheath was described by Schwann as a thin structureless membrane covering the myelin nerve sheath. He stressed the fact that it is a distinct entity and not a part of the connective tissue "neurilemma." The latter term was used by Bichat in 1802 to describe the epineural and perineural connective tissue which he recognized macroscopically. Objection has been raised especially by Plenk to this distinction between the connective tissue neurilemma and the Schwann sheath. The "Plenk sheath" is simply the Schwann sheath. The problem can be solved only by the application of embryologic methods and not by the study of adult tissue. The medullary or ectodermal origin of the Schwann sheath is now generally accepted.

The term "neurilemma" has been used in various ways and to include various structures (Key and Retzius; Marchand, 1880; Hyrtl; Cornil and Ranvier; Stöhr). The confusion is increased by referring to the immature Schwann cells as "lemmoblasts." They are not indifferent "blasts" nor do they originate from Bichat's "neurilemma." The tumors of the endoneurium and perineurium are classed as fibroma. The terms "neurilemma," "neurilemmoma" and "lemmoblastoma" should be dropped to avoid further confusion with Schwann cell tumors.

There is also no advantage in the use of special terms to describe the mature and the immature forms of Schwann cell tumors. A multitude of analogies are at hand.

Since the differentiation of embryonic nerve cells cannot be directly observed, any classification of tumors derived from them can at present be only preliminary. There are some useful classifications of the normal cells. The portions of these dealing with the sympathetic or autonomic nervous system are applicable to the present discussion. One of these by Bailey and Cushing is a typical model.

- Indifferent migrating crest cells
  - 1. Bipolar neuroblasts (ganglion cells of dorsal root ganglion)
  - 2. Sympathicoblasts
    - a. Unipolar neuroblasts, multipolar neuroblasts
    - b. Sympathetic ganglion cells, chromaffin cells
    - c. Capsular cells

Globus (1940) classified the normal cells as follows:

- Neural crest
  - 1. Neuroblast-sympathoblast
    - a. Chromaffin cells
    - b. Sympathetic ganglion cells
  - 2. Schwann cells

The terms used for the tumors of these cells correspond. Hett (1925) separated the sympathoblasts and the pheochromoblasts within and without the adrenal glands. This is of typographic rather than cytogenic interest. Foot (1940) distinguished the cellular and fibrillar tumors of the nerves from those of the nerve sheath but stressed the degree of differentiation of the tumor cells as did Pick and Bielschowsky. The following classification, similar to that of Foot, is based on histogenesis and uses the terminology of pathology.

CLASSIFICATION OF NERVE TUMORS

Normal Cells	Tumor
Mesodermal	
Perineurium, Endoneurium	Fibroma (combined with nerve tissue or nerve tumor); Endoperineural fibroma
Ectodermal	
A. Cerebrospinal Nerve System	
Neuroblast	Neuroblastoma
Neurocyte	Neurocytoma
Neurocyte: ganglion cell	ganglioma
Neurocyte: neurofibril	neuroma
Neurocyte: nerve sheath cell	neurinoma
Neurocyte: glia cell	glioma
ganglioma + neuroma	ganglioneuroma
neurinoma + neuroma	neurinoneuroma
B. Sympathetic Nerve System	
Sympathogonia	Sympathogonioma
Sympathoblast	Sympathoblastoma
Pheochromoblast	Pheochromoblastoma
1. Sympathoblast: ganglion cell	ganglioma, paraganglioma
1. Sympathoblast: neurofibril	neuroma
1. Sympathoblast: nerve sheath cell	neurinoma
2. Pheochromoblast	Pheochromoblastoma
pheochromocyte in adrenal and "paraganglion"	pheochromocytoma; "paraganglioma"

Fibroma originates from perineurium or endoneurium, the mesodermal portions of the nerve. It is not neurofibroma since it does not contain nerve fibrils. It may be combined with another type of tumor to produce, for example, neuroganglio-fibroma or neurinofibroma.

That part of the classification dealing with the cerebrospinal nervous system is self explanatory. Certain difficulties arise, however, in classifying the tumors of the sympathetic nervous system. The youngest indifferent sympathetic cells have been said to differentiate to both nerve cells and chromaffin cells (Wiesel, 1905). The indifferent *Bildungszellen* (building cells) of Wiesel were called sympathogonia by Poll, and these were said to give rise to sympathoblasts and pheochromoblasts. The term "sympathogonia" is not generally accepted by embryologists since the chromaffin cells are not nerve cells, although in their immature stages they are intimately associated with the sympathoblasts and they migrate together into the adrenal gland and elsewhere. The pheochromoblasts are not blasts, capable of differentiating in more than one direction, but are really pheochromocytes and can mature only to chromaffin cells. The tumor of these cells should, then, be known as pheochromocytoma or chromaffin cell tumor. Its cells may vary in degrees of maturity within a single tumor, as Bielschowsky (1932) himself recognized. There is little value in accepting his recommendation of dividing these tumors into pheochromoblastoma and pheochromocytoma. Similar circumstances of variations of maturation are known to occur in many tumors.

The mature Schwann cells in the sympathetic nervous system arise from the immature Schwann mother cells and are the same as those of the cerebrospinal nerves which give rise to neurinoma. But no instance of neurinoma of the medulla of the adrenal gland has been described, although this is a frequent site of the immature type of ganglioneuroma. The nerves of ganglioneuroma are described as covered by many Schwann cells, and these may even show evidence of proliferation.

It should be noted that many of these tumors do not reproduce pure stages corresponding to those of normal development. This complicates the problem of classification. The differentiation or maturation of the tumor cells may not be similar throughout the tumor at any given moment. Even when the tumor closely resembles normal tissue, the cells are not identical with the normal ones.

In spite of these difficulties, the general principles of the classification given will be useful. An attempt has been made to simplify it. Insufficient as this simplified classification may appear at first glance, it seems more useful for teaching purposes and less confusing.

SIMPLIFIED CLASSIFICATION OF NERVE TUMORS

Normal Cells		Tumor
	Mesodermal	
Perineurium, Endoneurium		Fibroma (Endoperineural fibroma)
	Ectodermal	
	A. Cerebrospinal Nerve System	
Neuroblast		Neuroblastoma
Neuroblast	ganglion cell	gangliocytoma
	neurofibril	neuroma
	nerve sheath cell	neurinoma
	glia cell	glioma
	B. Sympathetic Nerve System	
1. Sympathoblast		Sympathoblastoma
Sympathoblast	ganglion cell	gangliocytoma
	neurofibril	neuroma
	nerve sheath cell	neurinoma
2. Pheochromocyte		pheochromocytoma

It will be noted that in the simplified classification cells of equal capabilities in the central and the sympathetic nervous system are described by similar terms. The term "blast" describes an indifferent element capable of differentiating into two or more different types of cells ("cytes"), whose developmental capabilities are limited and fixed in one direction. The indifferent sympathoblasts are the equivalent of the neuroblasts of the cerebrospinal nerves. They are sympathetic neuroblasts.

It is true that the normal indifferent elements, the blasts, undergo some visible changes before they differentiate. This is only an indication of aging. The same or similar evidence of maturation is encountered not only in tumors whose cells have lost the ability to differentiate but also in the differentiated cytes of normal and tumor tissue. It would be generally useless and disturbing to apply a variety of terms to the different stages of maturation in tumors composed of cytes or blasts. The neuroblasts may be more or less mature but remain neuroblasts and should not be called neurocytes before differentiation to ganglion cells. Thus in the simplified classification neurocytoma is eliminated. On the other hand, cells which are recognized as differentiated, i. e. incapable of differentiating into other types, are termed cytes and not blasts. There is then no blastoma of chromaffin cells, Schwann cells or glia cells, and these tumors are termed respectively pheochromocytoma, neurinoma and glioma (gliocytoma). The word "ganglion" really means a nodule, and a ganglion is produced by a proliferation of cells. Gangliocytoma might, then, be preferable to ganglioma.

It may also be pointed out here that fibrous regression of nerve tumors does not justify the use of the terms "fibroma" and "neurofibroma." Many types of tumors undergo such regression. Connective tissue fibrils may proliferate, become hyalinized and even replace the original tumor. Similarly, normal nerves or ganglion cells and Schwann cells which do not participate in the tumor proliferation are occasionally seen to be surrounded by and included in the tumor.

Glial tissue, and even neuroepithelium, is occasionally found in tumors of the peripheral nervous system and especially in ganglioneuroma (Risel) and in metastases (Jacobsthal). Many authors have ascribed this to a local shift of cells during embryonic life. This concept presupposes a transportation of some cells from their original location to another as a result of some form of violence during embryonic development. Such dislocation of cells by so-called illegal cell connection happens often but relatively seldom results in formation of a tumor (R. Meyer). It is known that embryonic cells (blasts) possess potentialities which may occasionally develop in unusual places. The potentialities of embryonic cells are not normally all utilized and selective development of them is usually limited to the requirements of particular local functions.

Many types of nerve tumors are generalized, involving the whole nervous system, as is particularly seen in von Recklinghausen's disease. By comparison the nerve tumors of the generative tract are much more frequently single tumors. Likewise they tend more often to be simple in structure.

Nearly every type of nerve tumor that occurs elsewhere is occasionally found in the pelvic structures. Several types of nerve tumors will be omitted from the present discussion. In ovarian teratoblastoma undifferentiated nerve tissue belonging to the central nervous system may sometimes overgrow the remaining teratomatous tissue and produce fatal metastases. Sacrococcygeal teratoma sometimes involves the urogenital apparatus of the newborn female. These and some other mixed tumors will not be discussed.

SYMPATHOBLASTOMA

That the medulla of the adrenal gland is identical in development and structure with some prevertebral sympathetic ganglions is supposed to be known.

The sympathoblastic tumors are similar to the embryonal stage of the adrenal glands. The cells therefore often resemble lymphocytes. The cytoplasm develops fibrils and typical rosettes (Küster; Schilder; Wright; Scott, Oliver and Oliver). The cell fibrils may be grouped to bundles which form a sort of waist (Wright). This, however, has not often been observed in sympathoblastoma, and it occurs in other tumors. Round cells in nerve tumors are not necessarily sympathoblasts. Immature stages of neurinoma have a similar appearance, as will be discussed later in this article. In some cases the cells have shown evidence of maturing as in normal tissue. The cytoplasm develops fibrils, and rosettes are formed with their central portions filled by a network of fine fibrils (Küster; Schilder; Wright; Scott, Oliver and Oliver).

Earlier investigators considered fibrillar structure to be characteristic of glioma. But since Herxheimer pointed out that the fibrils in the adrenal tumor may be impregnated with silver and since Edith Buelbring demonstrated a similar impregnation of fibrils in a very immature intra-abdominal neoplasm diagnosed as neuroblastoma gangliocellulare, it has been generally accepted that sympathoma is a tumor of nerve tissue and is not glioma. Other investigators have agreed (Rinscheid; Butz). References to other literature dealing with tumors of the adrenal medulla are given. Wells described these tumors in detail.

Sympathoblastoma in the pelvis is rare. It has been known to occur in the bladder in children of both sexes. One tumor of this type occurring in the bladder of a girl 26 months old was described as neuroblastoma (Wolbach and Morse). Another (Harvey and Tennant) was found in a girl only 1 month old.

Sympathoblastoma seems to be extremely rare in other portions of the female genitalia. A growth of this type from a 27 year old woman was described as *sympathoma uterin* (Lemeland and Durante). This only partially mature tumor was not identical with "sympathoma embryonale." Some similarity to the ganglioneuroblastoma of Robertson must be admitted, although ganglion cells were not present.

A large tumor from the pelvic region of an infant 6 months old was described by Rocher as "an embryonic sympathoma." On biopsy it was diagnosed as fibroma. It seemed to be inoperable and radiotherapy was given, after which the tumor shrank and became calcified. The child was in good health two and a half years later. There would seem to be little justification for the use of the term "embryonic sympathoma" in this case.

A paravaginal tumor was described by Sturgis as "neuroblastoma (medullary blastoma sympathogonioma)." The photomicrographs do not show sympathogonia but do show rhythmic structure (i. e. palisade formation) and, in part, embryonic cells. This will be considered under the heading "Neurinoma."

GANGLIOCYTOMA

Law and Robertson described the same tumor, which was adherent to the coccyx of a 16 year old girl. Its characteristics would place it between sympathoblastoma (immature) and gangliocytoma (mature). Robertson called it ganglioneuroblastoma. The main part of the tumor was composed of "sympathogonia and sympathoblasts," while two small nodules showed unmixed gangliocytoma. Similar tumors have been found elsewhere, and the cell types may be mixed or may occur in groups of similar cells as in Robertson's tumor. This is the only one described as occurring in the pelvis.

GANGLIOCYTONEUROMA

The term "ganglioneuroma" is applied to tumors composed of ganglion cells and nerve fibrils without regard to the locality. The terms "paraganglioneuroma"

and "peripheral gangliocytoneuroma" have been used to describe similar tumors of peripherally situated nerve ganglions. Such tumors arise not only from neuroganglions but also from single or scattered neurocytes.

Wegelin collected descriptions of 25 cases of ganglioneuroma arising in various parts of the sympathetic nervous system. About the same number of cases of pelvic ganglioneuroma have now been described. Attention has been called by McFarland and Sappington to the striking variation of the relative content of ganglion cells, neurofibrils and "neuroganglia" in these tumors.

Lewis and Geschickter included under "paraganglioma" all varieties of sympathoblastoma and all chromaffin tumors. It might be noted here that although the neuroganglions of Frankenhäuser contain single and small groups of chromaffin cells (Penitschka), pheochromocytoma does not seem to have been encountered in this region.

Gangliocytoneuroma in the Hilus Ovarii.—The tumors thus designated appear more as hamartoma and hamartoblastoma. They differ in structure from pelvic gangliocytoneuroma in that they are almost entirely composed of proliferating ganglion cells, the nerve fibers, or neurofibrils, proliferating to a lesser degree.

Figure 1 *A* is from a tumor in the mesovarium and hilus of an ovary of a 67 year old woman who also had adenocarcinoma of the body of the uterus. Nerve fibers could be seen entering the base of the tumor near the rete and were scattered through the entire tumor between the numerous ganglion cells. The ganglion cells of the tumor were of irregular polygonal shape or pointed or club shaped. The cells were sometimes single and sometimes arranged in groups or cords. In one area of the outer zone, the cells were multinucleated, and the nuclei were lying close together in the form of a syncytium. The cells here had endofibrils (fig. 1 *B*) which were connected to nerve fibrils. The nuclei were large, round and stained equally except for occasional evidence of chromatolysis. This tumor was diagnosed as gangliocytoneuroma of the peripheral ganglions or paraganglions. In other parts of the hilus and mesovarium a few ganglion cells in association with normal nerves and "interstitial cells" were found.

Small nodules in the same region and somewhat resembling the tumor just described have been reported by Wallart, Neumann and Joachimovits. These nodules did not contain giant cells and to some extent resembled hypertrophic peripheral neuroganglions. I have frequently seen similar small groups of nerve cells in the mesovarium and the broad ligament, particularly in newborn infants. It is remarkable that tumors of these cells are so rare and that they remain so small. This might be due to a lack of embryonal cells.

Nerve fibers were not conspicuous in the small nodules just mentioned. In the pelvic retroperitoneal neurocytoma, on the other hand, nerve fibers predominate.

The large masses of peripheral paracervical and paravaginal neuroganglions do not give rise to gangliocytoma. In this regard they are even more sterile than the parovarian neuroganglions in spite of the fact that they are present in the paracervical and in the paravaginal tissues in overwhelmingly larger numbers. There is no adequate explanation for the absence of tumor formation from them.

Gangliocytoneuroma of the Uterus.—Single ganglion cells in the uterus have been described by some investigators, but their presence is denied by others. In 1912 I described a subserous ganglion on the uterus of a newborn child.

Borst mentioned a case described by Rindfleisch as one of fibroma of the uterus. The tumor was largely made up of spindle cells but contained ganglion cells. Borst found nonmyelinated nerve fibers in this tumor and called it amyelinic neuroma.

Fingerland and Sickl described a polypoid tumor from the cervix of a 41 year old woman. It measured 3 by 3 mm., was covered by columnar epithelium and contained some glands. It was made up of Schwann cells, numerous ganglion cells and many nerve fibers, some medullated and some passing through ganglion cells. There is insufficient description of the Schwann

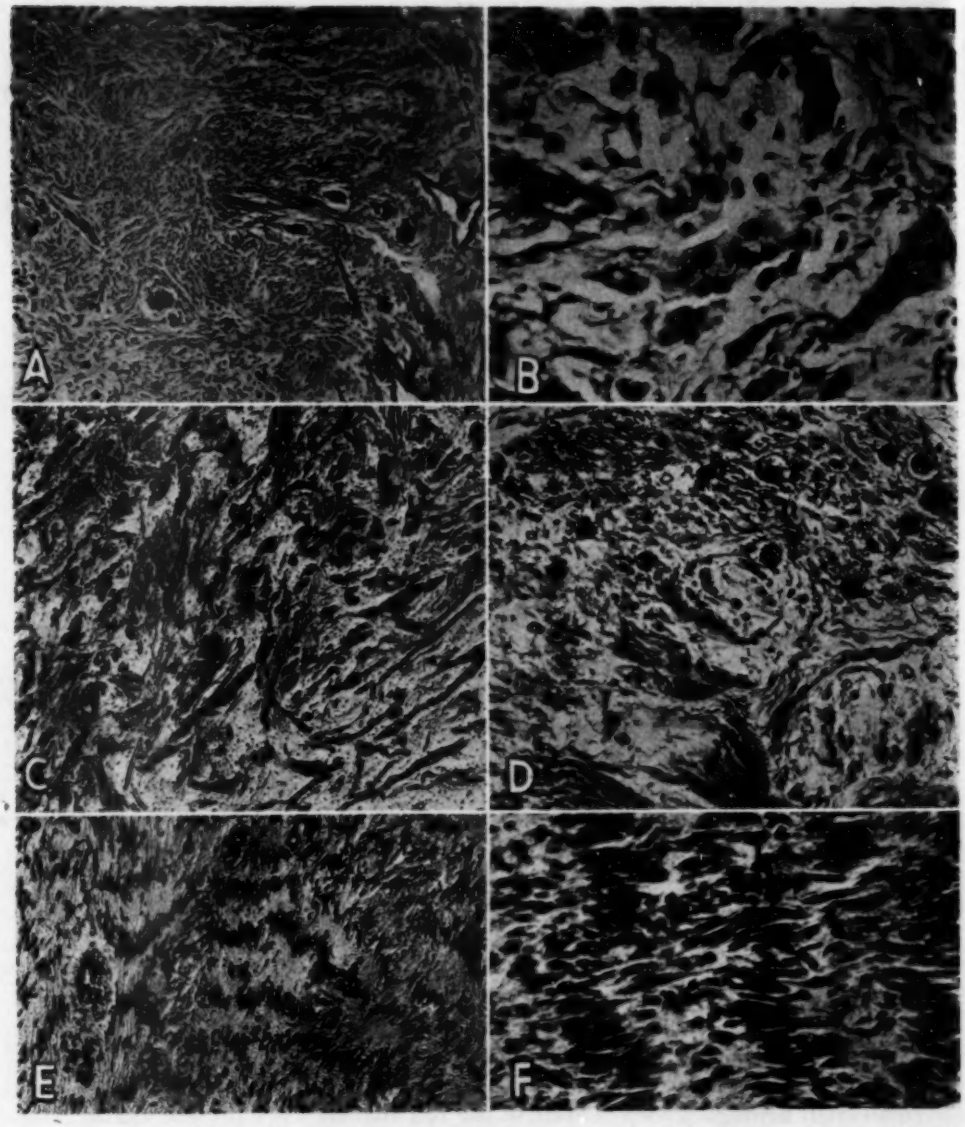

Fig. 1.—*A*, paraganglioma; section from a small specimen in the hilus of an ovary of a 67 year old woman.

B, section from the same tumor as *A*. Some of the ganglion cells contain endofibrils. Many cells are multinucleated.

C, gangliocytoneuroma from the pelvis of a 19 year old nullipara. Nerve fibers are seen in bundles like nerves but are without perineurium.

D, section from the same tumor as *C*. There is a diffuse scattering of ganglion cells.

E, advanced stage of development of rhythmic structure due to elongation of the fibrils in a regressive area of a tumor diagnosed as myoma.

F, neurinoma of the uterus.

cells but it is conceivable that they might accompany nerve fibers without themselves proliferating, as they do in the mature adrenal tumors. No note is made of rhythmic structure (palisade formation).

Pelvic Gangliocytoneuroma.—Retroperitoneal tumors in the pelvis are most often mature gangliocytoneuroma. It is peculiar that most of them are noncancerous. It is possible, however, that some retroperitoneal cancers have not been recognized as tumors of nerve tissue.

Approximately 20 cases of pelvic "ganglioneuroma" have been described.[1] Three of my own are described later in this section. Most of the patients were young women. Nerve tumors of all kinds occur in the bladders of young children and especially in those of boys but gangliocytoneuroma of the pelvis seems to be rare in men. One case of gangliocytoneuroma of the pelvic sympathetic trunk of a 4½ year old boy has been described by Farmer and Linell.

Sympathetic gangliocytoneuroma arises most often on the left side of the anterior surface of the sacrum and is tightly fixed to it. Occasionally it has a similar relation to the coccyx. In some instances it has been erroneously termed paraganglioneuroma, apparently because it extended into the broad ligament. The specimens described have for the most part been from patients in the third decade of life. There are, however, specimens from patients 6, 14, 17 and 19 years of age. Only 2 have been reported from patients beyond 30 years of age, and these patients were 32 and 38 years old.

Pelvic gangliocytoneuroma is frequently large. It filled the pelvis in a 6 year old child (Segato) and in 3 other patients (Strada; von Salacz; Vaicu and Popa). It was bilateral in 2 cases (Breitung; Neumann). It may be firmly attached to the sacrum, the local nerves and the rectum, and this may give rise to serious difficulty in surgical removal even though the tumor may be encapsulated. Frequently profound shock occurs during surgical removal and may even last for some days thereafter. The tumor may complicate pregnancy (Foster), and obstructed labor has been reported in 7 cases.[2] Three of the women died undelivered. Two were delivered by cesarean section, and one of these died. Operative removal has been successfully performed during pregnancy once (von Salacz) and during labor twice (Sippel; Meyer, case described in later paragraphs).

In most instances the tumor presents the histologic characteristics of mature ganglioneuroma. Ganglion cells are usually not numerous, but in view of the size of the tumor the total number of ganglion cells which it includes is certainly abnormal, and they cannot be considered as accidental inclusions in tumor tissue of unrelated origin. This conclusion is further supported by the finding of immature ganglion cells.

Many of the ganglion cells undergo regressive changes and necrosis. In the smaller specimens, where this is not advanced, the proportion of ganglion cells may be expected to be larger. Stoeckel described a tumor from a 19 year old girl which is the only one in which the ganglion cells were present in such large numbers that they appeared to crowd one another.

1. Beneke; Chiari; Cripps and Williamson; Schorr; Breitung; Law; Robertson; Stoeckel; Sippel; Strada; H. O. Neumann; Vaicu and Popa—2 cases; Haven and Weil; Segato; Fels; Rosenstein; Kehrer; von Salacz; Turunen; Evans and Francoma; Foster, Berman and Compton.

2. Beneke; Breitung; Law; Robertson; von Salacz; Sippel; Vaicu and Popa; Kehrer. Law and Robertson reported the same case.

The frequent occurrence of regressive changes in the ganglion cells is of importance in understanding neuroma. The ganglion cells have been described as short lived (Beneke; Wegelin).

The ganglion cells may be round, ovoid or star shaped. Where they lie close together, they may become polygonal. Two or more nuclei are frequently seen, though this is not necessarily a pathologic characteristic and is known to occur in the normal sympathetic ganglion (Penitschka; Beaton). The cytoplasm is homogeneous, without endofibrils or dendrites, perhaps as a result of compression by the actively proliferating nerve fibrils (Stoeckel). A few short projections from some cells (Neumann) and ganglions with axons and amyelinic nerve fibrils (Evans and Francona) have been reported.

The tumor is usually largely made up of amyelinic nerve fibers with few connective tissue fibrils. In some instances, however, the neurofibrillar tissue is at least in part interwoven and mixed with proliferated collagen fibrils to form a diffuse fibroma-like mass similar to neurofibroma of the skin. In pelvic ganglioneuroma the fibroma-like structure is of no genetic importance and is only evidence of tumor regression.

Three cases of gangliocytoneuroma of the female pelvis have been studied by me. The histories are not contributory, and the menstrual cycles were regular and normal. The patients' ages were 19, 22 and 24 years. The first tumor was from a nullipara, was the size of an orange and was located on the anterior surface of the sacrum (fig. 1 *C* and *D*). The second, from the patient aged 22, operated on just before delivery of her first child, was slightly smaller than the first and was likewise removed from the sacrum. The third tumor, about the same size as the first, was removed from the anterior surface of the coccyx. This patient had completed one pregnancy two years before. The clinical symptoms were as might be expected and included a sense of weight in the pelvis, constipation and dyspareunia.

In these three tumors the neurofibrillar structure predominated throughout. Relatively few ganglion cells were scattered about in small groups and were only loosely held between the fibrils. The small round nuclei of the ganglion cells may be seen in figure 1 *D*. Ganglion cells were seldom seen in the outer parts of the tumors but were usually found toward the centers, where the neurofibrillar structures were irregularly accumulated in heaps (fig. 1 *D*) and not in nervelike strands (fig. 1 *C*). In the outer portions of the tumors the fibrillar tissue was compressed into concentric layers.

The outer portions excluded, the 3 tumors presented some variations. The tumor of the 22 year old woman had a diffuse type of arrangement and contained more ganglion cells than those from the other 2 patients. The tumors of the latter (fig. 1 *C* and *D*) were composed of bundles of fibrils separated from each other by very loose connective tissue. In such areas the ganglion cells either were undergoing regression or were absent. Some of the better preserved ganglion cells were surrounded by garlands of nuclei. The difference in the histologic appearance of these tumors may be ascribed to edema.

The bundles were not identical with nerves in spite of their waving nervelike structure. They were not similar to the so-called plexiform neuroma, which will be considered later. As in other cases described in the literature, the nerve tissue in these 3 tumors had no perineurium surrounding the bundles. The nerve fibers were for the most part delicate and had many nuclei interspersed between them. These nuclei were very long and slender, although a few were more ovoid, clearer and surrounded by cytoplasm. Between the bundles and in the more dense areas the tumor contained a network of very delicate connective tissue fibrils interspersed with capillaries. Small vessels were everywhere present in considerable numbers but only a few large vessels were found. The nerve fibers were nonmyelinated. A few myelinated fibers were found in the tumor from the 19 year old girl.

In all 3 cases regression is for the most part softening or so-called mucinous regression rather than fibrosis. Some of the cells show chromatolysis of the nuclei. In others the cytoplasm is broken up so that it appears as a fine network. A striking change is that in which the cytoplasm became coagulated to form a dense mass which is darkly stained by hemalaun and hematoxylin.

Lymphocytes and some plasma cells were present in the edematous tumor tissue from the 19 year old girl. This is mentioned because Kehrer described a tumor in which round cells similar to lymphocytes were present in rows forming alveoli and glandlike structures. Some

of these were close to capillaries. From his description these might well have been lymphocytes. Lymphocytes do not show any evidence of differentiation to fibrils or ganglion cells.

Glia has been described by Risel in an abdominal retroperitoneal tumor in a child of 6½ years and by Jacobsthal in one in an 8 year old girl who also had metastases in the liver. Glia was not present in the 3 tumors which I have described. Kober described glia in a precoccygeal tumor. The original report of this is not available, but it and a similar tumor from the sacral region described by Jockel are apparently teratomatous neuroepithelioma.

The histogenesis of gangliocytoma and that of gangliocytoneuroma are likely to be the same. It is known that sympathoblastoma may change by differentiation into gangliocytoneuroma and that a part of the sympathoblasts may remain undifferentiated as in Robertson's "ganglioneuroblastoma."

It is not yet known whether gangliocytoneuroma always arises as a result of a proliferation of pathologic sympathoblasts (neuroblasts). It seems important, therefore, that palisading structure like that in neurinoma has not been described in mature or in immature sympathoblastoma or in partly differentiated gangliocytoneuroblastoma. The same is true of paragangliocytoneuroma. In the latter tumor it appears that the Schwann cells do not proliferate. The various descriptions do not give them particular attention. Evans and Francona described nonmyelinated fibers with many Schwann cells. These predominated and were enveloped by endoneural membranes. This finding suggests an accidental inclusion of nerves. In any event it is uncommon.

NEUROMA

Virchow's concept of "true neuroma," without ganglion cells, has been questioned by Beneke (1901), by Ribbert and by Pick and Bielschowsky. Gangliocytoneuroma may lose its ganglion cells by degeneration. Those who question the occurrence of pure neuroma point out that nerves should originate only from ganglion cells. This may apply to those types of nerve tumors which arise from congenital anomalies, but there is no good reason to doubt that pure neuroma may be formed from the peripheral nerves themselves. In neuroma most of the neurofibrils are amyelinic as in gangliocytoneuroma.

There is only one acceptable report of neuroma of the uterus (Kleine). This was described as "neuroma myelinicum" and was close to but well separated from a tumor termed "sarcoma fibromyochondroplasticum." Ganglion cells were not present. It was obtained from the uterine fundus of a 44 year old quindecipara.

"True neuroma," without other nerve components or fibromatous tissue, occurs in the vulva, the paravaginal tissue and the perineum. In tumors of this group the connective tissue forms only a supporting framework. Fibrous regression of a varying degree may, of course, be recognized.

Neurofibroma may occur in the pelvis and genitals either as a single tumor or as a part of generalized neurofibromatosis. Yamaura described generalized neurofibromatosis in a 22 year old virgin. The urinary bladder was involved in a tumor as large as a child's head. The neurofibromatosis involved the mucosa of the rectum, the ureter, the cauda equina and the vulva.

In some of the reported cases (Kehrer quoted Simpson and Kennedy) it is difficult to decide whether the tumors were to be classed as neuroma, neurinoma in part or only fibroma. It is probable that most of them contained neurofibers. Von Recklinghausen accepted the tumors in 2 other cases (von Walther and Okterlony) as being a part of generalized neurofibromatosis. One vulvar tumor which was a part of generalized neurofibromatosis also involved the thigh, the perineum, the vulva and the vagina and necessitated a cesarean section (Scharpenack and Albert).

Single neurofibroma of the labia, without von Recklinghausen's disease, has also been described by Bondi and by Maczewski. The so-called molluscum pendulum is sometimes neurofibroma.

A particular type of neuroma closely related to von Recklinghausen's disease has been called "neuroma cylindricum plexiforme" (Verneuil) or "plexiform neuroma and tendrillar neuroma" (*Rankenneurom*) (von Bruns). It is thought to be a congenital malformation (Courvoisier) and occurs particularly in the skin, sometimes generalized (Ouvry) and sometimes with production of elephantiasis. Occasionally it occurs in the abdomen (Baltisberger). Wegelin (1909 b) raised the question as to just what part the nerve tissue plays in this tumor or whether only the endoneurium and the perineurium proliferate. In some of the tumors occurring in the pelvis the nerve proliferation cannot be doubted. The nerves are lengthened and twisted to become convoluted and to produce thick cords and nodules which may form a continuous chain resembling a varicocele from the broad ligament through the paravaginal tissue to the labium majus (Schmauch). One tumor of this type (Pick) associated with von Recklinghausen's disease in a 26 year old woman arose from the region of the sacrum and had been present for sixteen years. Delivery was obstructed, and surgical removal was followed by sepsis and a fatal issue. The tumor showed sharply demarcated cylindric nerves with an irregular plaiting of loose connective tissue with darkly stained nuclei. In another (Bohnen) the nodules extended by continuity through the paravaginal and pararectal tissue and came close to the anus. The patient was 27 years old, a tertipara, and had last been pregnant five years before. The tumor showed no ganglion cells but only myelinated and nonmyelinated nerves.

It is worth noting that the tumors cited showed neither presence of ganglion cells nor proliferation of Schwann cells. The literature is reviewed by Strauss and by Baltisberger.

NEURINOMA

The term "neurinoma" (nerve sheath blastoma) was coined by Verocay to describe a tumor which he considered to arise from the Schwann mother cells. It is not a tumor of nerve fibers. Fibrous regression does not justify the term "neurinofibroma."

Verocay recognized the fact that Schwann cells, like ganglion cells and nerve fibers, are products of "neurocytes," but he did not confuse the fibrils of neurinoma with nerve fibrils. He also recognized the fact that neurinoma may be associated with other nerve and fibrous tissue tumors, and he referred to these as mixed tumors. The typical rhythmic (i. e., palisading) structure of neurinoma is the essential feature of Verocay's conclusions. Verocay pointed out that others (Heller; Genersich; Soyka) had previously recorded observations on this type of tumor.

Verocay showed that the fibrils of this special type of tumor lack the typical staining properties of connective tissue fibrils.

His concept of the histogenesis of the tumor was that it arises not from fully developed Schwann cells but from "neurocytes" after their differentiation into ganglion cells, nerve fibers and Schwann cells. While the Schwann cells proliferate to form the tumor, the other derivatives undergo regression. Some confusion and disagreement (Wegelin; Askanazy) have arisen in regard to this concept.

As more cases were reported, two main questions arose. These had to do with the usefulness of special staining and impregnation methods for the identification of the various components of the tumors and the reliability of the palisade-like structure as characteristic of a nerve sheath origin. The finding of collagen fibrils in neurinoma has entered the argument since it has been held that Schwann sheath cells do not produce collagen (Penfield). Collagen fibrils from the walls of blood vessels may be scattered through any tumor. It is likely that the collagen of the sheath of Key and Retzius (unfortunately now called the Laidlaw sheath) arises

from fibroblasts and that perineural fibroma also arises from connective tissue. The divergent concepts [3] as to the origin of argentaffin fibrils seems to overlook the fact that these fibrils are not a monopoly of either the ectoderm or the mesoderm. One must agree with Bailey and Hermann that the divergence of opinion has no point if both endoneurium and the Schwann sheath produce the same intercellular substance. Under any circumstances it must be admitted that the use of specific staining has thus far failed to clear up the problem of the histogenesis of neurinoma. Tissue culture experiments (Murray and Stout) suggest that the specific nerve sheath tumor originates from the Schwann cells since in culture these form collagen without the intervention of fibroblasts.

It should be pointed out that collagen fibrils are not found in newly formed neurinoma. Their presence later is of little significance in determining the histogenesis of the tumor. Too much importance has been ascribed to the content of reticulin and collagen in nerve tumors.

Ratzenhofer described a typical example of neurinoma in which he found lipoid granules similar to those of myelin and the granules of Reich. Such granules occur also in normal Schwann cells of myelinated nerves and are distinguished from those of cell regression by metachromasia after Feyerter's thionine staining. Myelinated nerves are not present in neurinoma. Ratzenhofer concluded that his findings supported Verocay's concept. There may be a connection between these findings and the statement of Geschickter that palisading does not occur in the absence of myelin.

Ewing traced neurosarcoma to "the Schwann cells in the neurinoma." This concept would suggest that Schwann cells may also give rise to tumors other than those with palisade-like structure. The possibility cannot be denied.

The rhythmically structured neurinoma is made up of nuclei and fibrils. The nuclei are for the most part long and waved or hook shaped. Each has a clear membrane and most often two nucleoli (Berblinger, 1918). One nucleolus and some granules have been described by Sommer. Cell boundaries are lacking, and the nuclei are scattered about in a trellised "syncytium" of thin fibrils. These plasmatic fibrils are not sharply outlined nor do they resemble glia (Krahn). In all descriptions, the main feature is the palisading of the nuclei in rows or layers. These alternate with rows of fibrils so that there is a certain rhythm. The fibrils are cytoplasmatic, as will be described later, and belong to the nuclei. This must be stressed. Palisades of similar appearance are produced by muscle cells. The rows may lie perpendicular to the long axes of vessels (Krumbein; Lauche), although this is not constant. This is due to the fact that the nuclei lie with their long axes parallel to the vessels. The rows are not often in straight lines but tend to produce a garland form or to appear as whirls, rosettes or similar structures (Freifeld; Krahn). These forms occur in noncancerous as well as in cancerous neurinoma (Wallner).

Neurinoma may show other types of structure or may show these combined with the pure palisading form. This variation is due to various types of regression, to the fact that the tumor is in an early stage and at least partly made up of embryonal cells or to the presence of cancer with its immature and atypical cells in diffuse proliferation.

Transitions between the usual noncancerous and the cancerous type of neurinoma have been recognized. These have been called "sarcoma" without

3. Foot, 1927; Nageotte and Guyon, 1930, a and b; Mallory and Parker; Penfield; Laidlaw; Masson, 1932; Nageotte.

regard to the ectodermal origin of the Schwann cells. Ewing stated that in neurogenic sarcoma the predominant part played by the Schwann cell is evident since Stewart demonstrated that the specific cells and structures of the tumor in the early stages are confined within the sheaths.

Regression may occur in both the noncancerous and the cancerous form of neurinoma. Liquefaction is seen. Its early stages are manifested by gaps between the fibrils. More advanced stages (Antoni) may lead to actual excavation, the cavities containing a tough colloid-like material. In the regressing areas the rhythmic structure may be lost (Sommer).

Antoni distinguished two types of neurinoma. Type A is made up of a procession of palisading bipolar nuclei of equal length alternating with fine fibrillar substance. Type B is characterized by rounded cells of unequal size scattered through a reticulum of fine interlacing fibrils. Occasionally the cytoplasm is visible. It is evident that Antoni was dealing with two stages of the same tumor. Type A represents the culmination of tumor growth and development, while type B appears to be an earlier stage with evidence of partial regression. Similar findings in one of my tumors will be described later in this article.

In a specimen of neurinoma described by Masson (1932) the palisading was absent in some areas. He suggested that there are two different organogenetic properties of the Schwann cells, one for sensory and one for motor nerves. However, one finds areas of palisading contiguous with other areas where it is absent, and this would involve the assumption that the tumor has developed from both types of nerves. It seems clear that the rhythmic structure occurs in tumors of nerves of both types. Any type of tumor with specific structures will include areas where these are absent. All tumors show similar variations in appearance as a result of regression. This is important in the diagnosis of tumors.

Until 1925 the palisade-like structure was considered to be an exclusive characteristic of neurinoma and, as such, diagnostic. However, similar structure has been found in the musculature of some organs and especially in uterine myoma.[4] In myoma the palisading is most often seen in areas which are in regression. There are, however, also special tumors of the uterine wall which show rhythmic structure throughout.

Genesis of Rhythmic Structure.—Many investigators have given attention to this problem. Francini, Verocay and others have held that it is an inherent quality of Schwann cells. Masson (1932) supported this opinion by comparing the structure of the tumor with that of a tactile corpuscle of the skin. In his figure 12, however, there is no palisading. A somewhat similar condition occurs in epithelial tumors when the cells of two rows touch one another at their free borders and the nuclei are layered at the basal portions. All theories directed toward an inherent rhythmic quality in specific tissue must take into consideration the fact that other tissue, especially that of myoma, also shows rhythmic structure.

Many other views have been expressed. Durante and Lemeland and also Verocay suggested that the cells might divide longitudinally. Krumbein suggested that a time factor in the form of rhythmic cell division might result in rhythmic structure. Others (Oberndorfer; Krumbein; Nestmann) expressed the thought that the rhythmic structure found in regressive areas of musculature might be due to contraction and hyalinization. Lauche expressed the belief that there are two different causes for the palisading of the nuclei. One of these, rhythmic cell division, is primary. The other is mechanical, following regression of the muscula-

4. Krumbein; Lauche; Geisler; Nestmann; R. Meyer, 1930; Opitz; Gerlach.

ture, and is secondary. The palisading in those portions of myoma that are undergoing regression has also been ascribed to mechanical pressure (Nestmann; Gerlach).

Comparison of younger and older stages leads one to the following concept. The palisade-like arrangement is not found primarily in the early stages of tumor cell proliferation (fig. 2 *A* and *B*) but is dependent on the gradually developing polarity of the nuclei. It increases with the elongation of the fibrils. The fibrils originate not from intercellular substance but from the cytoplasm. Even though this is not

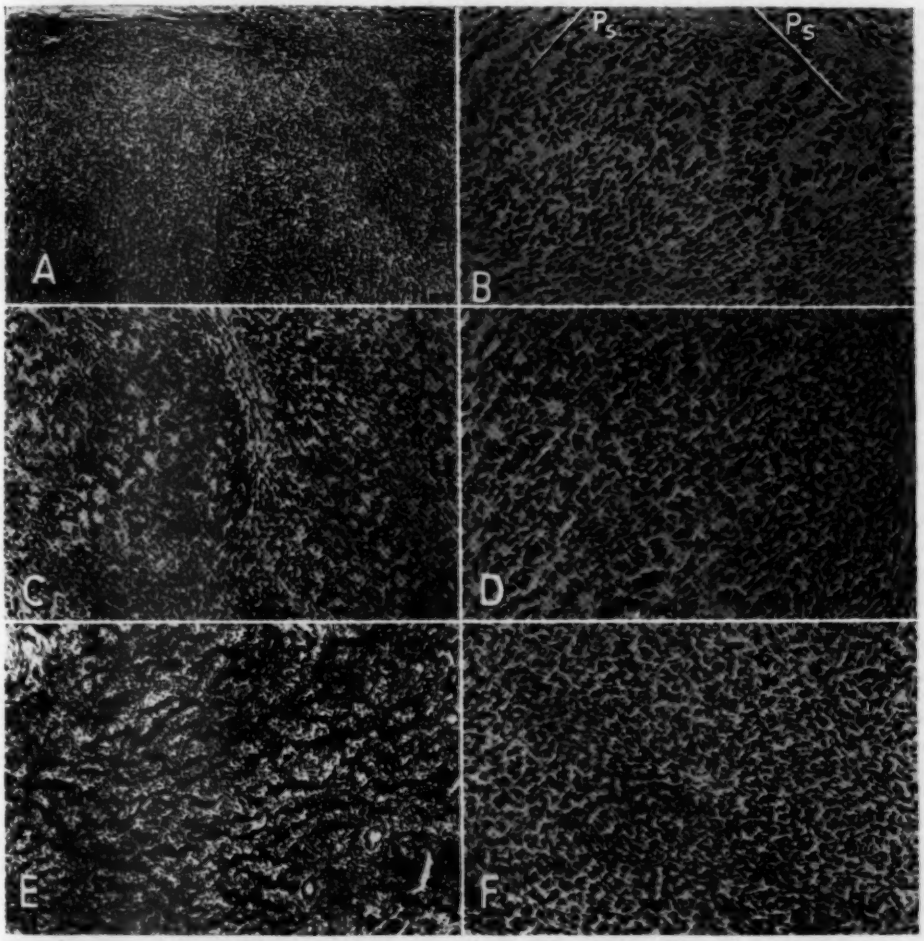

Fig. 2.—*A*, neurinoma destruens of the uterus. The tumor was a large one which perforated the uterine wall. This section is from a peripheral area.

B, section from the same tumor as *A*, showing pseudorosettes (*Ps.*) with cells containing hyperchromatic nuclei in a regressive area. No neuroepithelium is present.

C, rhythmic structure from the same case as *A* and *B*. Pseudoglomeruli are formed by the crossing of very short rows of nuclei.

D, section from the same tumor as *A*, *B* and *C*.

E, neurinoma of the uterus with typical rhythmic structure.

F, retroperitoneal abdominal neurinoma destruens with rhythmic structure of short nuclear rows similar to that seen in the uterine tumor (compare with *D*).

clear in all cases, one gets the impression from careful observation that definite bundles of fibrils belong to each nucleus. Much the largest number of these are on the two poles of the nuclei. The alternating layers appear as the fibrils lengthen. It is likely that the factors forcing this arrangement start and proceed mainly from the fibrillar portion. The large quantity of fluid which accumulates between the fibrils seems to be the causal agent rather than the tissue itself. The fibrils between laterally contiguous nuclei do not lengthen; the long sides of the nuclei remain close together. The polarized fibrils lengthen with steady accumulation of fluid. This presses the more solid nuclei closer together and forces the rows apart. The nuclei are then forced to divide in the long axis.

The palisade-like structure then appears to be dependent on a dynamic relation between the expansion of the fibrillar layers and the consistency of the nuclei. This might be described as a difference of aggregate states. In myoma and myosarcoma an adequate difference develops as a result of regression, liquefaction or hyalinization. In neurinoma this difference is attained in the process of growth through polarization of the nuclei and lengthening and liquefaction of the fibrillar zone, typical of the maturer stage of neurinoma and in greater degrees symptomatic of regression.

Palisade-Like Structure in Myoma, in Myosarcoma and in Neurinoma of the Uterus.—Rhythmic structure in large or multiple areas of typical myoma has been observed in 3 of our cases, reported by my associates (Opitz; Gerlach). I have observed a few similar cases since these reports and, in addition, a case of myosarcoma with rhythmic structure.

Degrees of palisading may be distinguished on the basis of the distance between the rows of nuclei. This is dependent on the stage of the process.

In a specimen of intramural myoma about 6 cm. in diameter, many areas were rich in nuclei. Other areas were softened, and while they stained poorly, the fibrils were readily recognizable (fig. 1 *E*). The nuclei had disappeared from the rows of fibrils. Transitions between the regressive areas and the regular myomatous structure were readily seen in many other areas.

A tumor with rhythmic structure diagnosed as myosarcoma was mentioned in a foregoing paragraph.

The patient was a 48 year old tertipara who complained of vaginal bleeding. At operation a tumor the size of a fetal head at term was found in the posterior wall and fundus of the uterus. Large portions of the tumor were merely myoma. Elsewhere sarcoma myocellulare was invading and destroying the myomatous parts. The rhythmic structure was found only in the sarcomatous portions, and here regression with softening was present. The sarcoma was in direct contact with completely degenerated portions of the tumor. The general picture differed only quantitatively from that seen in myoma with rhythmic structure. In the sarcomatous portions the rows of nuclei were most irregular, and the layers of fibrils contained more nuclei in consequence of the more active proliferation; the fibrils were short.

A similar case of myosarcoma of the uterus with rhythmic structure which I have identified was demonstrated by Brakemann. The tumor had involved the parametrium and the ovary on one side. Some portions of the tumor were myomatous and others sacromatous. Some of the sarcomatous growth was diffuse while in other parts it showed the typical palisade arrangement.

Neurinoma of the Uterus.—The question arises as to whether all uterine tumors with palisade-like structure are of the same origin. My associates and I have found 4 uterine tumors which differ in three cardinal points from those previously described. The rhythmic structure was not localized but was found throughout. The tumor cells had no similarity to muscle cells either structurally or in staining characteristics. The palisading (fig. 1 *F* and 2 *E*) was not confined to areas in regression but appeared wherever the nuclei were crowded. These, then, represent

a special type of uterine tumor. One of the 4 tumors was clinically a cancer and will be described separately.

The others were much softer than myoma on gross examination, and the color was more of a dirty gray. There was no evidence of a special capsule. On histologic examination the tumor tissue was not sharply demarcated from the uterine musculature but clearly invaded it. The nuclei were spindle shaped or blunt and were crowded in rows and garlands which alternated with layers of delicate fibrils. The rows of nuclei were, for the most part, broader than the layers of fibrils since the latter were short. In some areas the rows of fibrils were broader than the adjacent rows of nuclei. The thickness of the rows of nuclei sometimes changed suddenly since three or four nuclei piled irregularly together were sometimes immediately adjacent to an area where the thickness of the row was that of only one or two nuclei. Parts of nuclei were scattered between the fibrils.

The delicate plasmatic fibrils of these tumors were not sharply outlined as are collagenous fibrils. They were not stained by Van Gieson or Mallory stains. The fibrils ran toward the adjacent rows of nuclei, and as a group they lay together to form the layers which alternated with and paralleled the layers of nuclei. In contradistinction, glial fibrils became entangled on all sides of their nuclei.

There was at first some doubt as to whether or not these 3 tumors were noncancerous. Our first specimen, an intramural tumor 2 by 2 by 2.5 cm., somewhat resembled sarcoma (fig. 1 *F* and 2 *E*), although all of the tumor did not have such a wild appearance as these portions. The nuclei were of irregular shape and rich in chromatin and in most areas were crowded together. The fibrils were scarcely stainable, their arrangement was not quite regular and the fibril layers contained some nuclei. These findings in this and the 2 other similar tumors led me at first to consider them sarcomatous. Two of the patients, however, were well after nine months and the third after many years. Krumbein informed me that his 3 patients were also apparently cured after a considerable period. The tumors were then at least relatively benign. Neurinoma in other parts of the body is known to be in most cases noncancerous.

Neurinoma Destruens of the Uterus.—A 42 year old patient who had had three pregnancies ending at term and three abortions complained of a serosanguineous vaginal discharge. The uterus contained a tumor the size of an adult head. Small necrotic masses of it had been expelled through the external os. The tumor had broken through the uterine wall into the peritoneal cavity and the broad ligament and had extended to the pelvic wall and the rectum. There were some small nodules in the omentum, but no evidence of other tumors was found. The patient died two months after incomplete surgical removal (Gerlach).

On histologic examination some parts of the periphery were rich in nuclei and without any special arrangement (fig. 2*A*). The nuclei were elongated or irregularly polygonal and were rich in chromatin. Short fibrils were irregularly entangled about the nuclei. The diffusely arranged areas were less dense (fig. 2 *B*), and by liquefaction the nuclei had been arranged in irregular rows. The nuclei were rich in chromatin and were increased in size. They surrounded irregular cavities. These were not rosettes but pseudorosettes.

In other areas (fig. 2 *C* and *D*) the nuclei showed a variable arrangement in rows which crossed one another and thus superficially resembled the rosettes in sympathoblastoma of the adrenal gland. In the latter, however, the rosettes are quite irregularly distributed, tending to be single rather than in rows. In the tumor in question the young round cells of sympathoblastoma were absent. Neurinoma and sympathoblastoma should not be confused.

In this specimen of cancerous neurinoma the collagenous fibrils were present only about the vessels and in some septums. In all parts of the tumor the general character was the same, and no muscle cells were present. The short rows of nuclei (fig. 2 *B*) were not found in our other tumors of the uterus (compare figs. 1 *F* and 2 *E*). A more striking similarity was found in a retroperitoneal tumor diagnosed as neurinoma, which will be described presently. The uterine tumor in question is, then, to be considered neurinoma malignum. Large numbers

of uterine tumors diagnosed as sarcoma have been examined but there is no known description of a uterine tumor of this type.

Even so its seems important to confirm this diagnosis by reference to a very similar tumor which by its structure and retroperitoneal attachment to the posterior abdominal wall appeared without doubt to be neurinoma (Gerlach).

A woman 63 years old died five weeks after a laparotomy which showed an inoperable abdominal tumor. Autopsy revealed a tumor the size of a child's head. It lay retroperitoneally and was widely attached to the posterior abdominal wall with extension into the mesentery of the small intestine. The small intestine was adherent to the omentum, the bladder, the uterus and the large bowel. There were subserosal metastases to the uterus. Microscopic examination of the tumor showed a tissue rich in nuclei, similar to the tumor of the uterus described as neurinoma destruens. In both cases the nuclei were diffusely scattered but in many parts they formed rhythmic structures. As in the uterine tumor, the rows of irregularly shaped nuclei were short, with unsystematic changes in direction (fig. 2 *F*). The rows crossed one another, forming pseudorosettes. There was some development of delicate filaments, but nowhere were the fibrils longer than the nuclei.

The tumor just described corresponds to the retroperitoneal tumor termed "sarcomatous neurinoma" of Wallner, Brandes and others. This tumor and the very similar uterine tumor described on page 453 were of the same type and should be called neurinoma destruens to avoid misundertanding. Not fibroblasts but embryonal Schwann cells are proliferating and destroying.

The occurrence of accepted neurinoma destruens of the uterus supports the conclusion that a noncancerous form of neurinoma occurs in that organ. It seems to be even more frequent than the cancerous form. Three specimens of noncancerous neurinoma of the uterus have been described in the foregoing pages, the diagnosis of which was based on the rhythmic structure throughout the tumor, the absence of associated regression and the absence of muscle cells.

One other cancerous uterine tumor with palisade-like structure is described in the literature. It is the well known uterine tumor from a 2½ year old girl described by L. Pick as sympathoma embryonale. In Pick's figure 5 the rhythmic structure is typical of neurinoma. The remainder of the illustrations do not support the diagnosis of sympathoma. The "rosettes" (Pick's figs. 3 and 4) are crossed rows of palisades as in our figures 2 *C*, *D* and *F*. The rosettes of sympathoma are irregularly scattered. The nuclei in Pick's figure 3 are oval like Schwann nuclei but do not resemble the carrot-like nuclei of rosettes. The fibrils in true rosettes are quite irregularly crossed. The arrangement around vessels is not typical but the result of necrosis (Pick's fig. 6). The staining of the fibrils is the least reliable finding.

I cannot agree with Pick's diagnosis but must conclude that his case is one of neurinoma partially made up of immature Schwann cells. This is supported by the fact that no palisade-like structures have been found in sympathoma embryonale of the adrenal gland. The only similarity between sympathoblastoma and the immature areas of neurinoma lies in the fact that in both the cytoplasm changes into fibrils. The nuclei of embryonal Schwann cells are considerably larger than those of sympathoblasts. The latter are indifferent cells, and while they occasionally differentiate into ganglion cells and nerves, they do not produce palisade-like structures. Such structures in neurinoma are the product of immature cells specialized as are the Schwann cells and not capable of further differentiation.

It seems necessary to change Pick's diagnosis to neurinoma destruens of the uterus. This is the more important because sympathoblastoma arising from the female sex organs is not known and because sympathoblastoma, especially the paradigm of the adrenal medulla, does not contain rhythmic structure.

Neurinoma of the Pelvis and of the Broad Ligament.—Retroperitoneal neurinoma of the pelvis seems to be rare, especially by comparison with ganglioneuroma and with retroperitoneal neurinoma of the abdomen.

A tumor described by Fels as retroperitoneal unripe ganglioneuroma (sympathicoblastoma) in the pelvis is simple neurinoma. The illustrations show some waving of the fibrils and partial palisade arrangement. Some structures "similar to rosettes" are briefly mentioned but not illustrated. A few single cells are called ganglion cells in spite of the fact that they were

no larger than "sympathoblasts." Only one cell of this type is to be seen in Fels's figure 3. It has no similarity to the ganglion cell but is in regression. The whole tumor was undergoing fatty degeneration, as was the tumor in the following case of pelvic neurinoma. What has been said of Pick's case is apparently applicable here.

Krumbein described a typical specimen of neurinoma from a 49 year old woman. It occurred on the lateral pelvic wall and reached to the sphincter ani.

A tumor described by R. T. Frank as retroperitoneal cystic neurinoma was connected with the lower lumbar and the two upper sacral nerves. It contained medullated and nonmedullated nerve fibers and islands of palisading nuclei of Schwann cells.

Zagarese reported as neuronoma a giant tumor in the left broad ligament of a 41 year old woman. It was found five years after her third pregnancy. It had a dense, broad connection with the lower half of the posterior surface of the uterus which made subtotal hysterectomy necessary. The question arises as to whether the uterus was the original site of the tumor.

Paravaginal Neurinoma.—A case of neurinoma in the rectovaginal septum has been described by Xavier and Fialho.

A 49 year old woman complained of pressure on the bladder and rectum and some bleeding. The vaginal and the rectal mucosa in contact with the tumor were ulcerated. These parts were removed with the tumor. The tumor measured 7.5 by 5.5 cm. and contained some liquid-filled cavities. Histologically, there were rhythmic structure, fascicular areas and reticular ones, which were softened and showed no evidence of cancer. The pictures show unequivocal neurinomatous structure.

Two other cases of neurinoma have been described by Sturgis as instances of "sympathoblastoma" of the vagina.

The first was that of a 59 year old woman who had had four pregnancies. Her menopause had occurred ten years previously. Examination showed a tumor the size of a hen's egg arising from the left side of the rectovaginal septum and protruding into the vagina. Biopsy of the mass was followed by radiotherapy, which reduced the tumor to half its original size. The patient was in good health a year and a half later. No further change in the tumor occurred in this time. This tumor was not sympathogonioma or sympathoblastoma since Sturgis' figures 1 and 2 show a typical whorl arrangement of long spindle cells with large masses of fibrils attached to their poles. This resembles our figure 2 *E*.

The second case was that of a 32 year old quartipara, complaining of irregular menses. A tumor the size of a hen's egg protruded from the left side into the vagina. The tumor was incised and pale yellow masses removed. Radiotherapy was given, but the tumor again began to increase in size. Radium was applied, but six months later there was rapid growth with extension of the tumor. In her figure 4 a rhythmic structure may be recognized although the nuclei and fibrils are not as large as they become in later stages. In her figure 3 the nuclei are round and oval with irregularly distributed chromatin. There is evidence that the fibrillar cytoplasm has commenced to be tangled. This is similar to our figure 3 *A* but not so clear. Both tumors are neurinomas.

Partly Immature, Partly Mature Neurinoma of the Pelvis.—A tumor was obtained from a 39 year old patient who had had eight full term pregnancies. Her history included recurrent renal lithiasis and a recent rupture of the appendix with peritonitis. Nine months previously she had been admitted with anuria and severe lower abdominal pain due to bilateral renal calculi. During the next three months pelvic lithotomy was done on the right side and heminephrectomy on the left because of renal calculi with chronic renal infection. She became pregnant almost immediately thereafter, and because of the chronic renal infection abortion was performed at the twentieth week of pregnancy by subtotal hysterectomy. At this time a retroperitoneal mass about 8 by 8 by 5 cm. was found to overlie the right common iliac artery. The ureter lay anterior to it. The mass was just to the right of the promontory of the sacrum and had a pedicle which connected it to the vertebral canal through a small anterolateral opening the size of a lead pencil. The mass contained some clear brownish fluid and was encapsulated but adherent posteriorly. It was removed, but during this process the patient went into severe collapse, from which she recovered on transfusion of blood.

Histologic examination showed the tumor to have a dense sclerotic connective tissue capsule. In the outer loose fibrillar zone of the tumor itself there were small areas of tumor cells which appeared to be in good condition, while in the more hyalinized areas of the capsule the tumor cells seemed to have been choked. A similar condition was found in the inner layer of the capsule. The better preserved areas of the tumor (fig. 3 *A*) showed quite immature cells. Their nuclei were round and rich in chromatin. The cytoplasm was distinctly outlined. In some cells the cytoplasm was seen to become fibrillar, with fluid collected between the fibrils. The cell boundaries interlaced with those of neighboring cells and were lost. Here, the nuclei had begun to lengthen, and their chromatin appeared in small granules. The

fibrils showed beginning polarization. Figure 3 *B* shows further elongation of the fibrils in the long axis of the nuclei and accumulation of fluid between them. The nuclei approached each other, bringing their long sides closer together, and became further elongated. Figure 3 *C* shows beginning palisading of the nuclei into rows which alternate with the fibrillar layers.

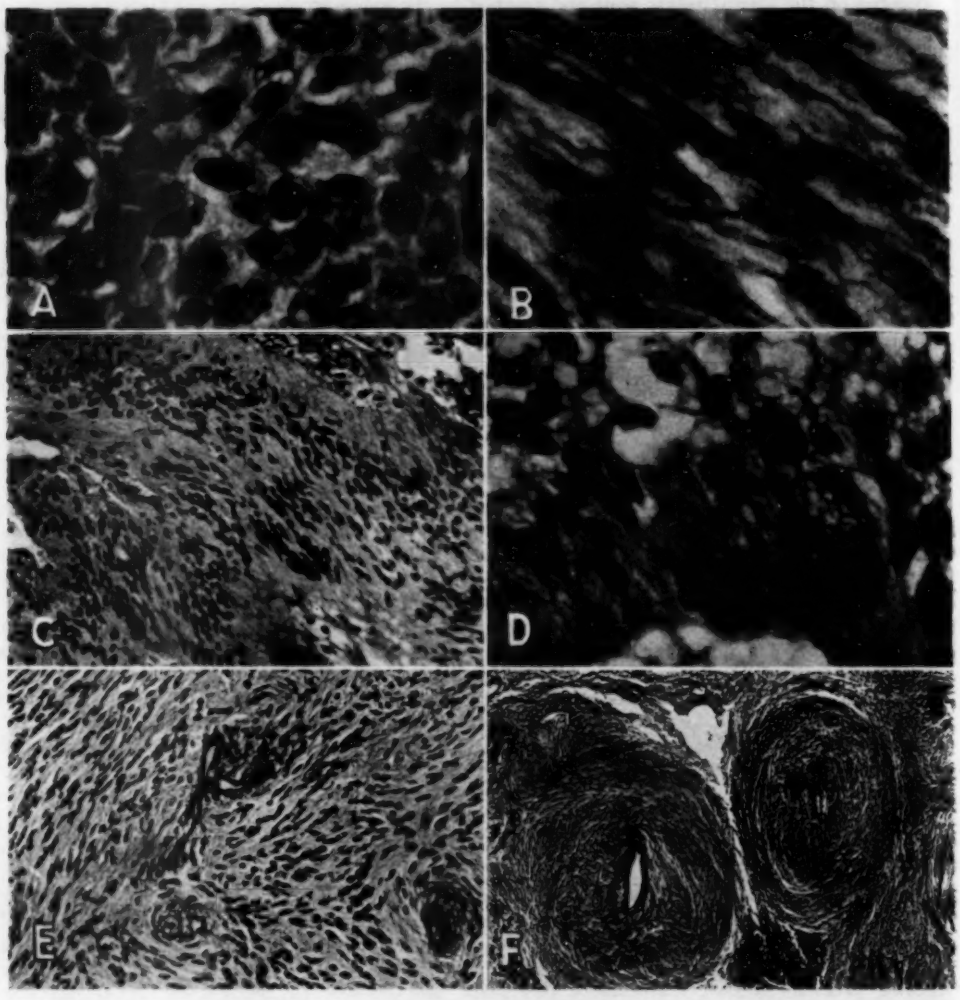

Fig. 3.—*A*, immature area of a pelvic tumor diagnosed as neurinoma. Some cells have round nuclei with unchanged cytoplasm; in others the cytoplasm has commenced to develop fibrils and to lose its outline. The chromatin is less dense in the elongated nuclei.

B, section from the same tumor as *A*. The fibrils are attached to elongated nuclei. They are loosened by fluid infiltration and are tangled. This is the beginning of rhythmic arrangement.

C, section from the same tumor as *A* and *B*. The nuclei are drawn nearer together and the fibrils are elongated. An irregularly rhythmic structure is produced. This is associated with regression, vacuolation.

D, section from the same tumor as *A*, *B* and *C*. The rhythmic structure is disturbed by liquefaction. Many small and some large cavities are present in the fibrillar layers.

E, section from the same tumor as *A* to *D*. A more diffuse, less rhythmic arrangement may be seen. Malformed vessels are present.

F, section from the same tumor as *A* to *E*. Tumor tissue surrounds the vessels in two layers.

The typical palisading was like that seen in other specimens of neurinoma. In most areas (fig. 3*D*), however, it was disturbed by regression and liquefaction. The coiled vessels in some areas (fig. 3*E*) showed hyaline degeneration of their walls, with no special arrangement of the neighboring tumor cells, which were diffusely spread throughout the tissue. These cells showed beginning chromatolysis and fading of the fibrils. In many areas this regression was in an early stage. The picture was similar to that of a special type of neurinoma which was mentioned in an earlier section as described by Antoni and also by Masson.

Another peculiarity of this tumor was the thick covering of tumor cells about vessels which were undergoing obliteration (fig. 3*F*). It suggested arteritis obliterans. The tumor cells were here arranged in layers, some of which were longitudinal while the outer layers were more circular. This suggested that the cells of the vessel walls were being replaced by tumor, a rare finding.

A third peculiarity was the lipoid degeneration of tumor cells throughout most of the tumor. First very small vacuoles appeared in the cytoplasm of the immature tumor cells. Vacuoles were seen also in the fibrils about the nuclei. Larger vacuoles developed until the tissue was converted into a clear mass of large vacuoles containing nuclei which were well separated from each other as in fatty tissue. Lipoid regression was seen mostly in the undifferentiated cells whereas the differentiated fibrillar areas showed more fading hyaline regression and liquefaction. In other areas hyalinized collagen tissue arising from the vessel walls replaced the tumor tissue. In this material there was no nerve tissue, argentaffin fibrils or nuclei, and silver impregnation was negative.

There is a remarkable contrast between this type of pelvic neurinoma, which does not contain other nerve elements, and pelvic ganglioneuroma, which is without any rhythmic structure.

NERVE TUMORS OF THE OVARY

These seem to be extraordinarily rare. A tumor of the ovary of a 50 year old virgin which was thought to be neurofibroma has been reported by Smith. The diagnosis was based on the finding of interlacing whorls of spindle cells. A detailed description is not given. The patient had von Recklinghausen's disease.

Stewart and Copeland mentioned a case in which multiple tumors of the skin appeared at about the time of the menopause. An ovarian fibroma was removed which seemed histologically to be typical "neurofibroma." These 2 cases may be identical. The question arises as to whether they are cases of neurinoma or of perineurofibroma.

A Case of Partially Embryonal Neurinofibroma of the Ovary.—The patient was 24 years of age and had had one normal pregnancy two years previously. Her past history was noncontributory. She complained of vaginal bleeding of six months' duration, but curettage yielded only normal proliferative endometrium. At operation a tumor 8 cm. in diameter was found to have replaced the left ovary. The tumor was irregular and rough in contour. Some areas were firm and others soft.

Histologically, the tumor was of similar structure throughout. No suggestion of teratomatous components could be found. There were a few small cysts lined by low serosal epithelium near the surface. The surface was covered by a simple dense fibrous capsule. The variety of tissue arrangement, the result of the presence of two types of cells, was surprising. There were embryonal round cells which when they occurred in maturer stages were rhythmically arranged. There were also spindle-shaped fibroblasts which served in part as supporting septums between the round cells (fig. 4*A*). They also proliferated independently, forming fibromatous tumor tissue between the palisade-like structures (fig. 4*F*). There was no common mother cell for the fibroblasts and the round cells. Even the younger fibroblasts were more mature than the round cells and were forming fibrils (fig. 4*A* and *B*).

When the individual round cell was immature it had a large darkly stained nucleus and relatively scanty cytoplasm (fig. 4*A*). The nucleus was a quarter or a third larger than a lymphocyte. This is of some importance in the differential diagnosis of sympathoblastoma or sympathoma embryonale, whose nuclei are of the same size as the lymphocyte. The immature cell had a sharp margin with a delicate cell membrane so that the cell was clearly separated from its neighbors (fig. 4*A*).

As the round cells increased in size (fig. 4*B*), the nuclei became clearer and the cytoplasm formed delicate fibrils. These at first lay inside the cells, but soon the cell boundaries broke up and the cytoplasmic ("tenuis") fibrils became entangled with those from neighboring cells

to form very fine filaments. The original extent of the individual cell could at first be recognized, since some of the fibrils of the cell membrane still demarcated an enlarged corona about the cell. This soon disappeared but the fibrils maintained their connection with the nucleus.

Neither the round tumor cells nor the fibroblasts provoked the formation of fibrils from any intercellular plasma. The fibrils of both were products of the cytoplasm of the two different

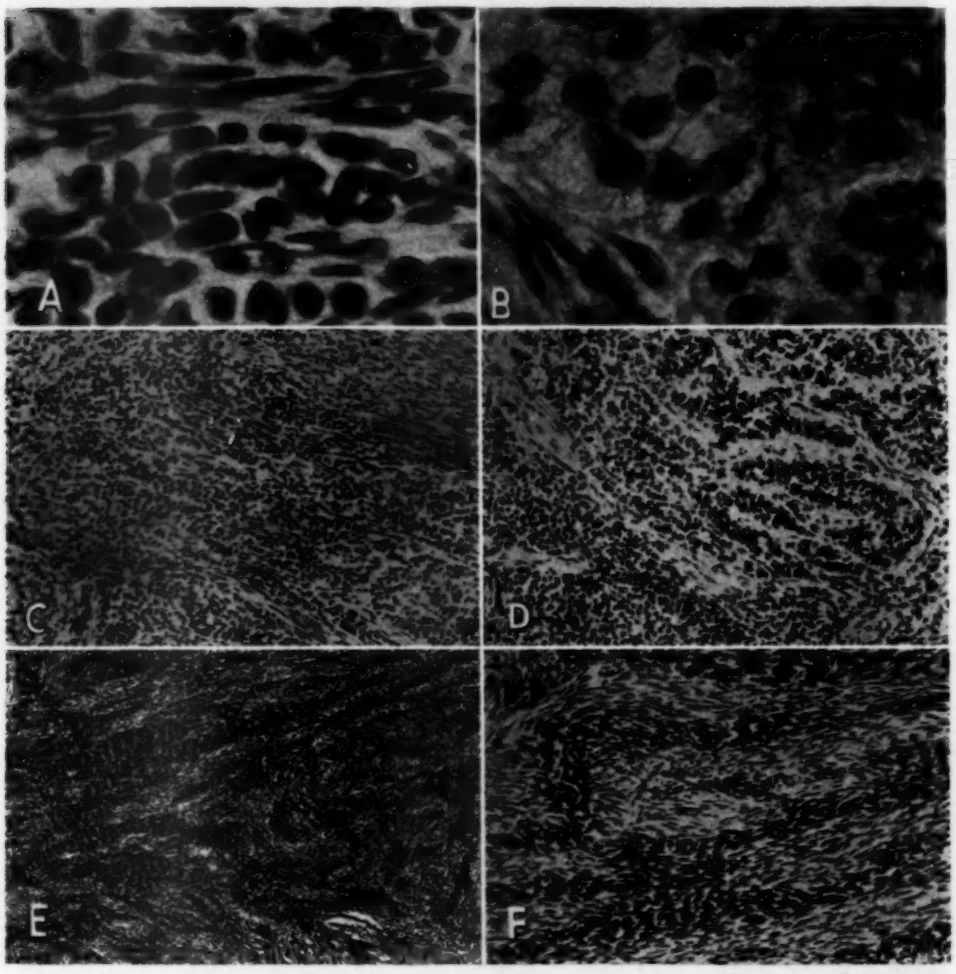

Fig. 4.—*A*, partially immature neurinofibroma of the ovary. Septums of long fibrocytes and capillaries separate rows and cords of round cells, the scanty cytoplasm of which is, for the most part, well outlined.

B, section from the same tumor as *A*. The cytoplasm of the cells has become looser and more fibrillar. Cell boundaries are still recognizable. After loss of these, the fibrils entangle. Note a fibrocytic septum to the left. The shape of the nuclei becomes irregular.

C, same growth under lower power. The same round cells as in *A* and *B* are seen between septums of fibroblasts, which may be in large masses (right upper area.)

D, section from the same tumor as *A* to *C*. As a result of early regression, hyperchromatic nuclei and epithelium-like cords are produced. The fibrillar connective tissue is edematous. A better preserved area appears to the left below.

E, section from the same tumor as *A* to *D*. Rhythmic neurinoma is irregularly interrupted by fibromatous tissue.

F, section from the same tumor as *A* to *E*. Fibromatous growth predominates over rhythmic neurinoma.

types of cells. As soon as the fibroblastic fibrils increased in size, they became stainable by eosin, Van Gieson and Mallory stains. The round cell fibrils were not stained by these dyes.

At first the round cells in the tumor proliferated diffusely. Later they were separated by the connective or the fibromatous tissue into groups, cords and even thin rows (fig. 4 *C*). In some areas (fig. 4 *C*, right lower area) the fibroblasts proliferated independently or at most included only a few of the round tumor cells. In some parts (fig. 4 *D*) the round cells were in epithelium-like groups, where they had some of the appearance of neuroepithelioma. This was present only where regression was occurring with hyperchromatosis and did not go on to the production of neuroepithelium, rosettes or glia. Regression was followed by necrosis. Similar areas were seen in the specimen of neurinoma destruens of the uterus (compare fig. 2 *B*).

Other groups and cords of the round cells changed by polarization and lengthening of the nuclei and fibrils into a palisade-like structure. Fibromatous structures were scattered through these areas (fig. 4 *E*). Here and there the palisade-like structure and elsewhere the fibromatous structure (fig. 4 *F*) predominated. But everywhere and in all stages of maturation the two could be sharply distinguished. The fibromatous tissue was more mature and had more clearly stained nuclei and more fibrils.

This, then, was a mixed tumor of the ovary, neurinofibroma. No consideration has been given to whether the fibromatous component arose from the endoneurium

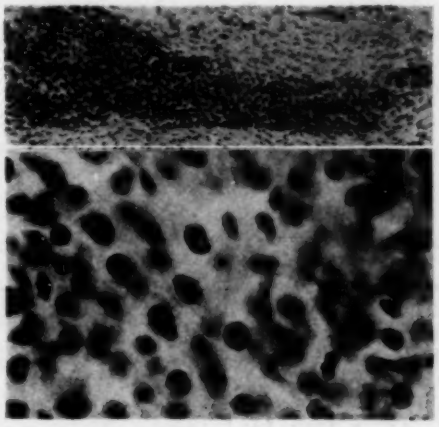

Fig. 5.—Microscopically small area in the labium minus consisting of round and some oblong nuclei whose cytoplasm is becoming fibrillar. Compare with figures 3 *A* and 4 *B*.

or from the ovarian stroma. It is well known that the ovarian stroma has the special ability of reacting to various tumors by proliferation.

This unique ovarian tumor was certainly not teratomatous. Not only were other teratomatous constituents lacking, but a wide experience with both teratoma and teratoblastoma has not brought to light findings which resemble the tumor under discussion. It had some similarity to one specimen of neurinoma of the sacrum (fig. 4 *D*). Both presented the same type of immature cells in large masses. At first individual cells were seen with round nuclei and well limited cytoplasm which later changed into fibrils. The rhythmic structure was a still later stage. This was not so far advanced in the ovarian tumor (fig. 4 *E* and *F*), and the fibrils were not so long as in some areas of the pelvic tumor (fig. 3 *C*). In this regard the ovarian tumor showed closer similarity to the uterine tumor (fig. 1 *F*). The diffuse areas in the sacral tumor were not fibromatous but neurinomatous structures and were dependent for some of their forms on local vascular conditions.

Immature Neurinoma of the Labium Minus.—An interesting specimen was found in the labium minus of a 26 year old woman who was accidentally killed. A club-shaped cellular area

was attached to a thin nerve bundle by a sort of handle (fig. 5). The nerve bundle could not be traced into the cellular area. The cellular area was rather sharply demarcated from the loose submucous connective tissue. Except for a few spindle-shaped connective tissue cells and two or three capillaries, the mass consisted of cells with round and ovoid nuclei. The cytoplasm in some cells was clear, but in most it was changing into fine fibrils which were beginning to connect with those of other cells. Some of the nuclei together with their fibrils were in tandem. This is similar to the young stages of neurinoma described in foregoing pages. The cells seemed to arise from a nerve. They were certainly neither connective tissue cells nor ganglion cells. There was no transition to neurocytes. The suggestion is that they are Schwann cells, and if this is so, the tumor is the youngest known neurinoma of the unripe type. It is not possible to carry out further examination of this tumor but attention is drawn to it in the hope that similar cases may be found and studied, particularly as to the identity of the type of nerve cell.

SUMMARY

Relevant features of the development of the nervous system are discussed.

A simplified classification of nerve tumors is presented and discussed.

The various nerve tumors which occur in the female pelvis and genitalia are described, and the literature is critically evaluated, with suggestions for correction of the diagnoses of the tumors described in some well known reports. New cases are added.

The problems which relate to neurinoma are detailed and discussed. The characteristic palisade structure of neurinoma is shown to occur also in uterine myoma. The palisading is not seen in very early tumors. It is due to accumulation of fluid between the elongated fibrils which arise from the two poles of the nuclei.

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BRONCHIOLAR LYMPHOID HYPERPLASIA AS A CAUSE OF EMPHYSEMA

REPORT OF A CASE

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Emphysema was described by Laennec,[1] and a theory was presented to explain its production by partial bronchial obstruction. Since that time various investigators have produced the lesion in animals, supporting Laennec's contention and adding to the general understanding of it. Today physicians recognize several types of emphysema[2]: (1) a type compensatory to other primary lesions; (2) an obstructive type with narrowing of the bronchi; (3) a senile or postural type, in which deformities of the thoracic cage are causal; (4) rare types, such as infantile familial emphysema with defective elastic tissue development and the secondary emphysema with Ayerza's syndrome, characterized by a vascular lesion with degeneration and fibrosis of lung tissue. We report a case in which emphysema was associated with bronchiolar stenosis caused by focal hyperplasia of lymphoid tissue. We have been unable to find a description of a similar case.

REPORT OF CASE

H. M., a 31 year old white man, an expert accountant, married, was first seen in June 1937 because of increasing shortness of breath. His illness might have been present in childhood, since it was reported that he had never been able to run and play with the activity and vigor of a normal child. The immediate history indicated that recurring attacks of excessive dyspnea (without associated fever) had been noted for the past three winters and that during the past year the patient had never been completely free from this symptom; there was a steady increase in the average dyspnea. The referring physicians had entertained the diagnosis of bronchial asthma, and he had been treated by injections and inhalations of ephedrine and epinephrine with moderate and varying success.

Past History.—In childhood he had measles, whooping cough, chickenpox, mumps and scarlet fever without known sequelae. He had always been susceptible to colds and had recurrent attacks of acute sinusitis. Tonsillectomy and submucous resection had been performed eleven and ten years before. He had spent two weeks in the Massachusetts General Hospital six years before for treatment of a febrile illness with transient splenomegaly and a blood picture that showed the aberrations considered to be diagnostic of infectious mononucleosis. Recovery from this illness with progression to a normal blood picture was considered to be complete. Some "nervous indigestion" during the past five years had been treated with diet, alkalinizing substances and carminatives; this disorder had not been disabling, and gastrointestinal roentgenograms with barium sulfate were reported to have shown only a spastic duodenal cap.

His habits were not remarkable; eating had been restricted only because of the supposed gastroduodenitis.

From the departments of pathology and allergy of the Roosevelt Hospital.

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The family history was of interest only in that the father suffered from "strawberry rash."

Examination.—The patient was well developed and well nourished. He was moderately dyspneic, breathing with a dry wheeze, and had an asthmatic type of cough. His temperature was normal. The head and neck showed nothing remarkable; the tonsils were out, the pharynx was slightly red and the left antrum transilluminated poorly. There were no palpable glands. The heart was in normal position, with sounds distant; the pulse rate was 80, with normal rhythm; the blood pressure was 120 systolic and 80 diastolic. Breath sounds were almost absent everywhere in the chest, with hyperresonance and normal fremitus; the contour of the chest was normal. The abdomen and genitalia and the skin were normal.

The urine, the stool and the blood were within normal limits on first and repeated examinations throughout the succeeding three years of observation. More than 2 per cent of eosinophils were never observed in the blood smears. The Wassermann reaction of the blood was negative on repeated tests.

Course.—The patient was studied extensively from the standpoint of possible allergic asthma and other cardiorespiratory disease. The course was intermittently downhill. The dyspnea was increasingly severe (with exacerbations lasting from a few hours to a few days), and there was only irregular relief from the usual therapeutic drugs employed in bronchial asthma, rest in bed and inhalation of oxygen (and oxygen and helium). During the three years of observation he had seven admissions to New York hospitals. Complete intradermal direct passive transfer tests with various inhalant and food allergens and with a number of bacterial substances (from cultures of sputum and of material from sinuses and bronchi) as well as elimination diets failed to reveal any significant sensitivity. Prolonged treatment by injection of autogenous bacterial preparations was of little apparent aid.

During the first year of observation roentgenograms of the chest showed small calcifications of healed, widely disseminated tuberculosis throughout the upper lobes of both lungs, some fibrosis, basal pleural thickening and generalized emphysema with definitely diminished excursions of the diaphragm. Bronchoscopic examination with instillation of iodized poppy seed oil 40 per cent (lipiodol) revealed a dry, reddened bronchial mucosa with no obstruction. Smears made from the bronchial mucus showed no eosinophils, and cultures were not significant. No abnormality of the bronchial tree was identified with the radiopaque material. Electrocardiograms showed changes indicative of bundle branch block and ventricular hypertrophy. These findings were subsequently verified. At the time of admission to the hospital in 1937 a diagnosis of tension pneumothorax on the left side was made mistakenly, and true pneumothorax on the left side followed an attempt to aspirate the pleural cavity; this resorbed and healed spontaneously without sequela.

In the second year of our observation the patient improved sufficiently to return to work for a few months. Roentgenologic study of the sinuses had revealed evidence of chronic disease of the left antrum, and radical antrostomy was performed on the left without event and without appreciable benefit. A thickened, pseudopolypoid membrane was curetted away, and pathologic examination of this disclosed nothing remarkable. With but one hospital admission, the patient had found some benefit from a self designed dehumidifier.

During the third year, studies of chest capacity indicated a total respiratory volume of 1,060 cc. at rest and 1,380 cc. after administration of epinephrine. Christie's sign, a staircase ascent of the spirometer tracing indicative of emphysema, was noted. A venous pressure of 8.0 cm. of water was measured. At this time the patient made a three month visit to the South and underwent treatment (with hospitalization) for three alleged minor pulmonary emboli. Inhalation of oxygen and ephedrine gave relief; this diagnosis could not be verified further, but the patient recovered sufficiently to return home. He then became worse, and the last seven months of his life he spent in the hospital, with increasing dyspnea at rest and progressively less relief from sympathicomimetic drugs and oxygen and helium inhalations. He died in a state of asphyxia in February 1940.

Pathologic Examination.—Thoracic viscera only were available for examination. Marked emphysema was demonstrated in both lungs with collapse in the lower lobes (fig. 1). Scattered patches and streaks of bluish gray color indicated mild anthracosis. The upper lobe of the left lung was larger than the lower and was pink-gray. The anterior borders were thick and rounded. The lobe was highly crepitant, and the margins were semitransparent. The lobules and alveoli were much enlarged. Cut surfaces revealed a uniform coarse honeycombed structure. The air sacs were considerably enlarged and were clearly visible throughout the entire lobe. The lungs had a feathery consistency. The bronchus and its divisions revealed no evidence of obstruction; the linings were smooth and pinkish red.

The left lung weighed 310 Gm. Nearly one half of the lower lobe was collapsed and had a peculiar spongy consistency. The upper half of this lobe was pale bluish gray; the pleural surface was smooth. The air spaces were greatly increased in size, one larger space

measuring up to 6 cm. in its greatest diameter. The walls were irregular and partial septums were present over them, giving the structure a ragged appearance. The air spaces were dry. The cut surface was pale and reddish gray. The cut surface of the upper portion revealed a finer honeycombed structure. Only a few of the spaces were greatly enlarged, and these measured at most 15 mm. in diameter. The bronchus with its smaller divisions was free of mucous plugs, and its lining was smooth and light red. Smaller divisions, to the lower portion and in the region of the larger spaces described, had dilated lumens at some points and much narrowed ones at others.

The right lung had large lobes, which were highly crepitant and feathery throughout. The lower portion of the lower lobe was dark red and was less crepitant than other areas. The anterior margins were round, pale and semitransparent. The cut surface of the lower lobe was dark red and moist. Small pieces cut from this floated high in water. There was a honeycombed structure, which was coarse in the lower portion. The upper and middle lobes were uniform in consistency and appearance, had rounded anterior margins and were highly crepitant and reddish gray. Their cut surfaces showed a fine honeycombed structure; the alveolar spaces were grossly enlarged. The bronchus and its divisions revealed

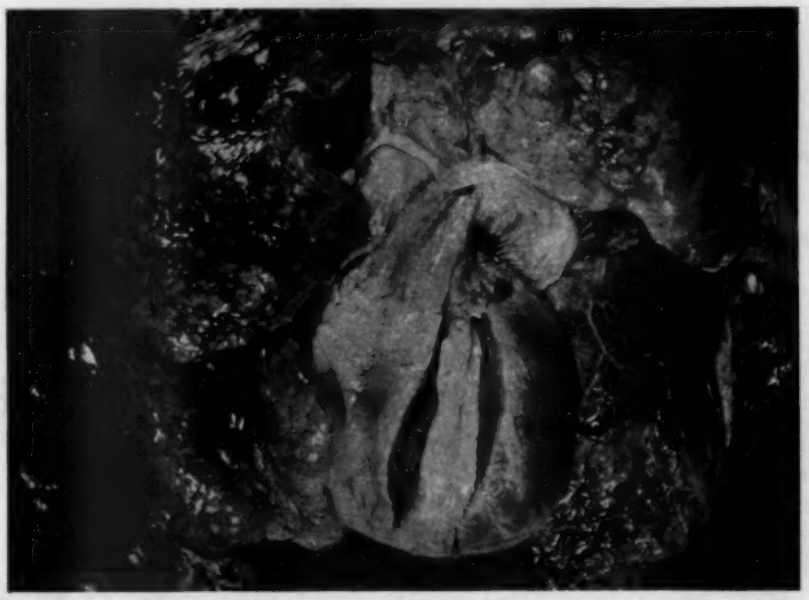

Fig. 1.—Thoracic viscera showing pulmonary emphysema and cardiac hypertrophy.

no evidence of obstruction. Small divisions of bronchi showed some variation in size; their lumens were not uniform in diameter. In some areas they were slightly wider in distal than in proximal segments. In subpleural areas a few scattered firm nodules, 2 to 3 mm. in diameter, were palpated. They were usually heavily pigmented (bluish black). The right lung weighed 520 Gm.

The peribronchial lymph nodes were not noticeably enlarged. They contained only a moderate amount of anthracotic pigment and revealed no notable change on their cut surfaces except an occasional firm gray area of fibrosis.

The thymus was not seen.

The heart weighed 450 Gm. The apex was blunt and was composed mostly of the right ventricle. The epicardial surface was smooth, with a moderate amount of fat present. The right chambers were large and dilated. Their linings were smooth, and they contained large postmortem clots. The foramen ovale was closed. The tricuspid valve measured 13 cm. in circumference, and its leaflets were thin and smooth. The pulmonary valve measured 9 cm. in circumference and was normal. The wall of the right ventricle measured 5 mm. in thickness at the apex and 10 mm. at the base. The myocardium of the right ventricle was uniformly firm and pale brownish red. The left auricle was not remarkable. The mitral valve measured 10.5 cm. in circumference, and the area along the closure line was some-

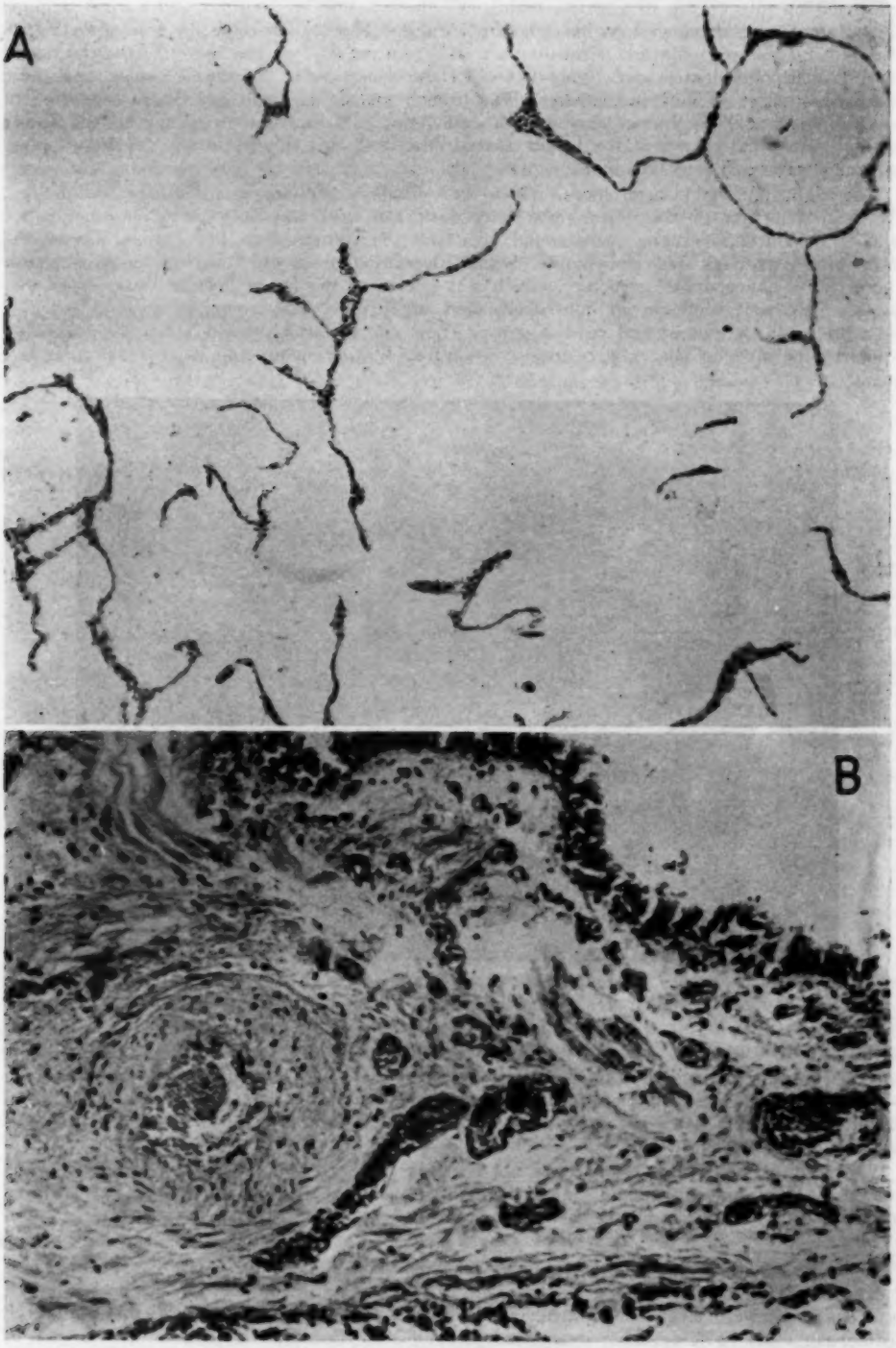

Fig. 2.—*A*, section of lung revealing thinning and rupture of alveolar walls. Hematoxylin and eosin stain; × 225.

B, section showing a bronchial wall with an adjacent arteriole, the lumen of which is almost obliterated by intimal sclerosis; no inflammatory cells are present. Hematoxylin and eosin stain; × 675.

what thickened. Its surfaces were smooth and grayish white. The chordae tendineae were smooth; two of them were slightly thickened and shortened. The lining of the left ventricle was not remarkable. Its wall measured 12 mm. in thickness at the apex and 18 mm. at the base. The aortic valve measured 8.5 cm. in circumference. Its leaflets were smooth; there was some yellow thickening at their bases. There were a few small gray islands present in the muscle of the left ventricle, which was pale and brownish red.

The orifices of the coronary arteries were widely patent. The main branches showed an occasional smooth yellow intimal plaque of small size with no noteworthy narrowing of the lumen. The ascending portion and the arch of the aorta revealed only an occasional small yellow intimal plaque and streak. The vessel was of normal size in these portions. Its wall

Fig. 3.—Multiple lymphoid follicles in and around a bronchiole. Hematoxylin and eosin stain; × 225.

was not thickened. The periaortic tissue was not unusual. The pulmonary artery demonstrated a prominent and dilated conus. The lining had scattered smooth pale yellow intimal plaques. They were present in moderate numbers and measured up to 1 cm. in greatest diameter.

Microscopic sections of lung showed emphysema everywhere. Areas where this was most pronounced revealed thin strands of tissue in which a few small spindle-shaped nuclei were present (fig. 2*A*). Spurs of broken alveolar walls were present along the longer segments. The alveolar walls in other areas were less thin and were made up of hyalinized connective tissue with few nuclei. No inflammatory cell infiltration was present. Slightly more stroma was seen in the region of blood vessels and bronchi. Widely scattered areas demonstrated macrophages containing brown and a few black pigment granules in their cytoplasm. These were seen in alveolar spaces and in interstitial tissue. Weigert's stain demonstrated elastic

tissue fibers throughout, stretched thin and diminished in some alveolar walls but nowhere entirely absent.

The bronchi showed thin walls of uneven thickness. The muscularis was variable in thickness but was not hypertrophied (fig. 2 *B*). The majority of the bronchi showed no cellular infiltration and no fibrosis except in widely scattered areas where a few lymphocytes could be seen. Smaller divisions of the bronchial tree were in some places considerably dilated, and this was most noticeable in those without cartilage. The mucosal lining was composed of a single layer of columnar cells. Mucus was scant, and the mucous glands were not hypertrophied; their alveoli were small. The basement membranes were not remarkable.

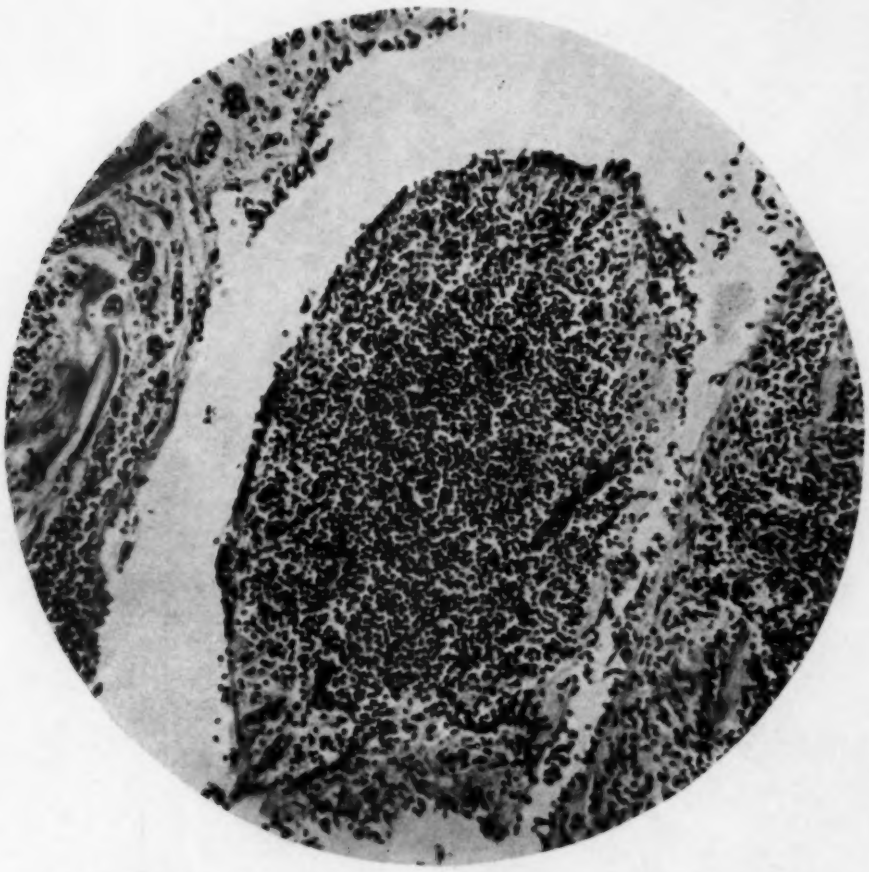

Fig. 4.—Pseudopolyp filling a bronchiole. Hematoxylin and eosin stain; × 675.

A most unusual histologic feature seen in the sections was lymphoid tissue, mostly in association with the small bronchi and bronchioles. It was seen in all the sections from both lungs (fig. 3). These collections formed lymph follicles as well as masses of closely packed lymphocytes. Large pale central areas were formed by medium-sized and larger cells, and these centers were surrounded by the smaller lymphocytes. This lymphoid tissue was situated in the walls, frequently bulging into the lumens of the small bronchioles. Such masses often formed polyp-like projections into the lumens, and these pseudopolypi caused varying degrees of luminal narrowing (fig. 4). When such a bronchiole was cut longitudinally, one might find multiple sites of narrowing between which the lumen was dilated. These collections of cells were composed entirely of lymphocytes. The lymph follicles were infrequently associated with arteries and veins but were most commonly associated with the smaller air passages. Small collections of closely grouped lymphocytes were rarely seen in the alveolar walls.

Sections from bronchial lymph nodes near the hilus showed mild anthracosis, little hyperplasia and small follicles.

The walls of many of the smaller pulmonary arteries and of the majority of the small arterioles were greatly thickened (fig. 2*B*). In some the media was hypertrophied, while in others intimal sclerosis was conspicuous. Larger branches revealed moderate degrees of intimal fibrosis with occasional vacuolation and a few recently formed thrombi grafted over areas of intimal sclerosis. No inflammatory reaction was evident in association with these vascular changes.

The myocardium showed hypertrophy of the muscle fibers and minimal collections of brown pigment at the poles of nuclei. No necrosis or fibrosis was seen in a number of sections from different areas, including the base of the interventricular septum.

COMMENT

This patient presented an unusual clinical history of increasingly severe dyspnea which terminated in fatal asphyxia. The immediate cause was marked emphysema. The left bundle branch block indicated by electrocardiogram cannot be explained even after pathologic examination of the heart; this was not the right axis deviation seen in some cases of cor pulmonale.

Pathologic features were emphysema, aberrant growth of lymphoid follicles, sclerosis of small branches of the pulmonary arteries and arterioles, and hypertrophy and dilatation of the heart. Absence of the usual features of inflammatory states and of the changes seen in bronchial asthma (luminal mucous plugs, hyalinization and thickening of the basement membrane of the bronchial walls with hypertrophy of muscle fibers, dilatation and hypertrophy of mucous glands and presence of eosinophilic leukocytes) [3] is of interest.

The hyperplasia of aberrant lymphoid follicles appears to have been the cause of the emphysema, with obstruction (and some ball valve action) of the small air passages. Normal human lungs do not show this type of lymphoid hyperplasia.[4] These lungs resembled the lungs of the rabbit and the guinea pig, in which lymphoid tissue is found in relation to the small divisions of the bronchi, more closely than other pathologic human lungs. In man varying amounts of infiltration by lymphocytes and hypertrophy of lymphoid tissue occur in areas of inflammation, in bronchitis, tuberculosis and such conditions, but in those conditions the hyperplasia is not diffuse and is not so intimately associated with the smaller divisions of the bronchial tree. In those conditions there are also present evidences of chronic inflammation, as fibrosis and diffuse infiltration by various types of inflammatory cells.

The origin and nature of this condition cannot be stated. There is no basis for suspecting inflammation, such as might be caused by inhalation of some mildly irritating substance, with selective stimulation of lymphoid tissue. The questionable entity of status thymicolymphaticus should be mentioned; the thymus was not enlarged, the mediastinal lymph nodes were not remarkable, and the aorta was of normal diameter. Other lymphoid tissues were not available for examination, but the peripheral nodes were not enlarged on clinical examination. There is little reason to classify this as neoplastic disease. One might consider a developmental anomaly in which the lungs contained increased amounts of lymphoid tissue such as that mentioned as present in the lower animals. This might have become hyperplastic under the influence of some irritant. A relationship to the antecedent infectious mononucleosis should be considered—could the ensuing disease be a residuum of that illness? This is doubtful since the patient's history suggested that there was difficulty early in life.

3. Macdonald, I. B.: Ann. Int. Med. **6**:253, 1932.
4. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1937.

We have been unable to find any report of an anomalous occurrence of this type of lymphoid tissue. Enlargement of the larger bronchial nodes with resulting stenosis and emphysema are reported; such cases are dissimilar and the causes have been apparent. Polypoid lesions in the larger bronchi are usually neoplastic, rarely lymphocytic, in type. One case of giant cell lymphoma with congenital cystic disease has been reported.[5] Long [6] described an interesting case of fatal emphysema of a giant bullous type and referred to others which had been reported. These cases showed progression of the disease to death, but the known and supposed etiology of each was unlike the picture seen here.

Miller [4] stated that he had not found lymphoid follicles in association with the respiratory bronchioles and that small collections of lymphocytes found distal to the second ventral branch (bronchus) are usually associated with the pulmonary veins and are not in the mucosa of the bronchial twigs. In persons 60 or more years of age the lymphoid tissue associated with the finer divisions of the bronchial tree is always increased; we have never seen this degree of hyperplasia in any age group.

The changes in the pulmonary artery and its smaller branches are those of atherosclerosis, intimal fibrosis and degeneration with secondary thrombosis. There is no inflammatory cell infiltration. Clinically, the Wassermann reaction was negative in several tests at different hospitals. Changes in the pulmonary arterioles, especially, offer a sound explanation for the cardiac hypertrophy (cor pulmonale). Parker [7] found right-sided hypertrophy in two thirds of his cases of emphysema, usually with unexplained left-sided hypertrophy as well. These vascular changes are usually thought to follow increase in pressure in the lesser arterial circulation. Despite much discussion as to whether emphysema precedes or follows vascular sclerosis,[8] it seems that in this case the emphysema occurred first and was the cause of increased tension with resultant changes in the blood vessels. The patient received large doses of sympatheticomimetic drugs. Epinephrine causes arterial changes, but in a case reported by Brooks and Kaplan [9] the pulmonary arteries were not involved.

SUMMARY

The clinical and pathologic records of an unusual case of fatal emphysema is presented. The pathologic features were aberrant lymphoid hyperplasia associated with terminal bronchioles, marked emphysema, sclerosis of pulmonary vessels and cor pulmonale. The obstruction of the small air passages by the lymphoid tissue offers an explanation for the genesis of the emphysema. The vascular sclerosis and the cor pulmonale are considered to have been due to the hypertension of the lesser arterial circulation which resulted from the emphysema.

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9. Brooks, H., and Kaplan, D. M.: Arch. Int. Med. **1**:329, 1908.

EXPERIMENTAL ATHEROSCLEROSIS IN THE CHICK

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Our previous experiments showed that atheroma can be produced in an omnivorous animal, the chick.[1] Lesions were produced in young White Rock cockerels by feeding cholesterol in cottonseed oil. The present investigation was undertaken to determine whether atheroma resulted from feeding the chicks cottonseed oil or from the marked underfeeding and undernutrition which had accompanied the cholesterol diet. In addition, it was proposed to observe the evolution of the vascular lesions with more prolonged cholesterol feeding.

White Leghorn chicks were selected for the present study since the adult birds are small and suitable for prolonged laboratory use. This also permitted exclusion of the possibility that cholesterol atheroma is peculiar to the White Rock variety previously used. While it is desirable to use chicks as young as possible in order to avoid confusion with spontaneous atherosclerosis, known to develop in older birds,[2] the high mortality of the very young (10 day old) chicks fed a cholesterol diet led us to select older chicks (14 weeks) for the present study. The period of observation was prolonged from fifteen weeks to six months. Cockerels were used again because of the higher incidence of atheroma reported in male birds.[3]

The exact site of atheroma was accurately observed for the purpose of comparison with the site of the spontaneous lesion expected to occur in these older birds. In birds generally, spontaneous atheroma is most marked in the thoracic aorta,[4] while in the rooster spontaneous intimal fibrosis with and without lipid and calcium deposits is found more often in the abdominal aorta.[5] In addition, intimal thickening has been described in the coronary arteries [6] and in the femoral and splenic arteries of the chick.[6a, b] The only reported spontaneous lesions in the heart were yellow plaques in the aortic valves of 2 year old hens.[6a]

PROCEDURE

White Leghorn cockerels 4 weeks old (tested for Salmonella pullorum infection) were obtained and kept in batteries in the laboratory for ten weeks for initial adaptation. During

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1. Dauber, D. V., and Katz, L. N.: Arch. Path. **34**:937, 1942.

2. Fox, H.: Bull. New York Acad. Med. **15**:748, 1939.

3. Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 6, p. 153.

4. Fox.[2, 3]

5. (*a*) Kesten, H. D.; Meaker, D. R., and Jobling, J. W.: Proc. Soc. Exper. Biol. & Med. **34**:818, 1936. (*b*) Uchiyama, T.: Virchows Arch. f. path. Anat. **277**:642, 1930.

6. (*a*) Kawamura, R.: Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose, Jena, Gustav Fischer, 1927. (*b*) Yamaguchi, M., abstracted, Japan. J. M. Sc. **2**: 143, 1925. (*c*) Fox.[2]

this time they were fed ad libitum on a commercial chick starter mash [7] supplemented with cod liver oil. Five chicks were lost during this initial period through cannibalism. The remaining 43, then 14 weeks old, were placed in individual battery compartments and divided into four groups. For this division, the birds were weighed and distributed so that each group contained small and large chicks (from about 800 to 1,700 Gm.) and had an approximately equal average weight at the start (fig. 1). In all four groups the basic mash diet supplemented with cod liver oil was continued and water was freely provided. The consumption of feed was measured by placing weighed quantities of feed in the bins of all the groups and deducting the weight of the residue from scrapings of the bins before discarding it and adding fresh weighed feed.

The first group, consisting of 10 cockerels, was fed ad libitum for the duration of the observation period as a control group.

The second, the group fed cottonseed oil, composed of 11 birds, was fed the described mash with added cottonseed oil ad libitum. The quantity of cottonseed oil and the method of

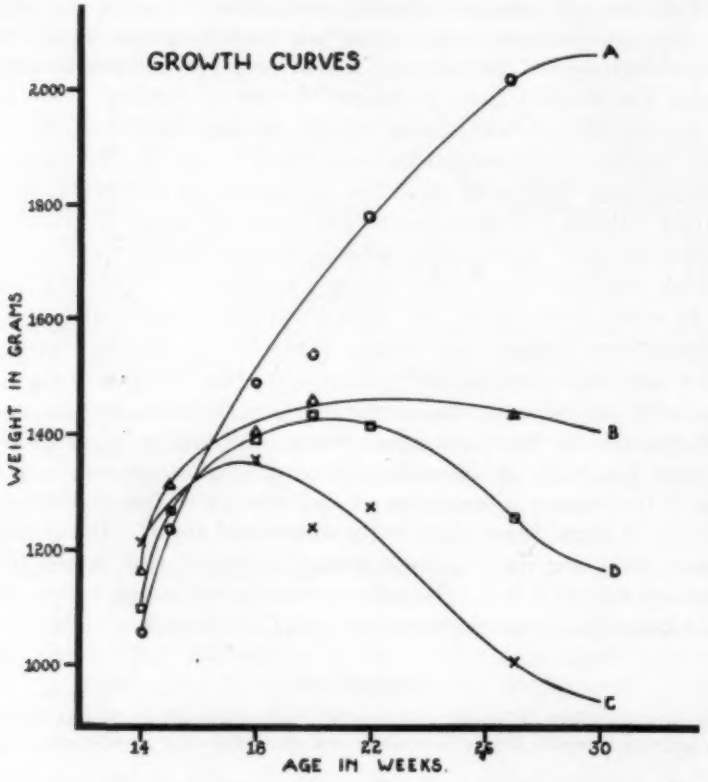

Fig. 1.—Growth curves of the four groups of cockerels during the period from the fourteenth to the thirtieth week of life: Circles designate the average weights of the controls (*A*), triangles the average weights of the chicks fed cottonseed oil (*B*), crosses the average weights of the underfed chicks (*C*) and squares the average weights of the cholesterol-fed chicks (*D*).

7. This mash was prepared from ground corn, wheat bran, wheat standard middlings, corn gluten meal, meat scraps, alfalfa meal, soybean oil meal, pulverized oats, fish meal, dried milk by-products, ground limestone, salt, manganese sulfate and fish liver oil. Cod liver oil U. S. P. was added (2.5 Gm. per hundred grams) to supply vitamin A. This mash contained not less than 18 per cent crude protein, 3.5 per cent crude fat, 51 per cent nitrogen-free extract (carbohydrates) and not more than 6 per cent crude fiber. It contained 1.4 per cent calcium, 0.75 per cent phosphorus, 0.0125 per cent manganese sulfate and 0.75 per cent sodium chloride. The vitamin A content was 1,000 units per gram and the vitamin D content 400 chick units per gram.

mixing were the same as used for the cholesterol-fed group (see next paragraph) in order to make the comparison of these two groups valid. The feed was prepared by mixing 20 cc. of cottonseed oil (Puritan oil, Proctor & Gamble Company) with each 100 Gm. of the commercial mash.

The third, the "underfed" group, numbering 11 chicks, served as a control for the total amount of mash consumed by the cholesterol-fed group.[8] These chicks received weight for weight the same quantity of dry mash as was consumed, exclusive of the cholesterol-cottonseed oil, by the cholesterol-fed group. Since this limited amount of mash was generally consumed in a day, their bins were empty for several days while the cholesterol-fed chicks were more slowly consuming their allotment. The feed of this group was replenished only when that of the cholesterol-fed group required renewal.

The fourth, the cholesterol-fed group, consisting of 11 cockerels, received 2 per cent crystalline cholesterol (Wilson Laboratories) suspended in cottonseed oil with the mash. This concentration of cholesterol was selected on the basis of our previous experience as compatible with prolonged study yet productive of lesions. Larger concentrations had been found to result in such marked emaciation as to curtail the duration of study. Cottonseed oil was used to improve absorption of the cholesterol. In preparing the feed for this group the dry mash was stirred into the cottonseed oil suspension of cholesterol in order to obtain a uniform mixture without lumping. Two grams of cholesterol in 20 cc. of cottonseed oil was mixed with each 98 Gm. of dry mash.

The development of all birds was followed. Each was weighed periodically to determine growth. Total blood cholesterol and cholesterol esters were determined without preliminary fasting. The Sperry-Schoenheimer method [9] adapted for photoelectric colorimetry was used on blood obtained from the alar vein. The survivors were killed at the age of 9 months.

Every chick was submitted to autopsy. The weight of the wet heart was measured by the technic employed for the rabbit in this laboratory.[10] The organs were examined grossly, and after fixation in solution of formaldehyde U. S. P. diluted 1 to 4, histologic sections were prepared from the thoracic and from the abdominal aorta, the femoral arteries, the heart muscle from the left ventricle and from lung, liver, spleen, kidney, adrenal glands, testis and pancreas and stained with hematoxylin and eosin. In some cases sections of the aortic and mitral valves, the femoral vein, the brachiocephalic artery, the stomach and the intestine were also examined. In addition sections of the aorta and the femoral artery were stained with the orcein elastic stain and with the Masson and Van Gieson connective tissue stains. Frozen scetions of aorta, kidney and adrenal gland were stained with sudan IV for lipid.

RESULTS

Survival, Growth and Development.—Intercurrent deaths during the period of experimentation depleted the number of chicks in all except the control group, from which only 1 bird was lost, at the age of 6 months. Two of the chicks fed cottonseed oil succumbed at the age of 5 months, 4 were lost at 6 months, and only 3 survived to the age of 9 months. Only 2 of the underfed chicks survived to the age of 9 months, 1 succumbing in the third month and 8 in the sixth month. Nine of the cholesterol-fed chicks succumbed at the age of 6 to 8 months, only 2 surviving to the age of 9 months. Because of this high mortality in the course of the experiment, 3 of the controls were killed at the age of 6 months to serve as controls for these chicks that died early.

The effect of the various diets on growth was followed only during the first half (three months) of the experimental period, since after that time the number of birds in each group except the control group rapidly diminished. As is shown by the average weight curves in figure 1, all four groups of chicks grew at an equal rate during the first experimental week. After that only the control group continued to grow at the same rate, reaching a plateau at the age of 6 months. The group fed cottonseed oil grew at a slower rate for six weeks, reached a lower plateau and tended to remain at this lighter weight. The underfed group grew even more slowly for four weeks and then gradually lost weight. The cholesterol-fed chicks grew at about the same rate as the cottonseed oil-fed group for six weeks and after that slowly lost weight.

Food consumption by the cholesterol-fed group was better in the present study than in the earlier one with younger cockerels [1] In the present experiment the cholesterol-fed birds consumed about three fourths as much as the control group fed ad libitum, and therefore this was the quantity of feed supplied to the "underfed" chicks. The group fed cottonseed oil

8. Through an error in recognizing sex, a pullet was included in this group.

9. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

10. Katz, L. N.; Sanders, A.; Megibow, R. S., and Carlen, S.: Am. J. M. Sc. **200**:731, 1940.

also consumed less than the control group and about the same amount as the other two groups. While the decreased intake of the cholesterol-fed birds may be due in part to the cottonseed oil used, our earlier observations [1] that dry cholesterol without oil was poorly consumed and was accompanied by loss of weight indicate that part of this decreased feeding is attributable directly to the cholesterol itself.

The cholesterol-fed cockerels tended to have a smaller comb and wattles than the birds of the other groups. In the exceptions in which the comb was large, it became pale and yellowish after several months of cholesterol feeding. The feathers of cholesterol-fed chicks appeared dirty, greasy and matted.

TABLE 1.—*Total Blood Cholesterol and Percentage of Cholesterol Esters*

	Control Group		Group Fed Cottonseed Oil		Underfed Group		Group Fed Cholesterol	
Weeks on Diet	Total Cholesterol, Mg. per 100 Cc.	% Cholesterol Esters	Total Cholesterol, Mg. per 100 Cc.	% Cholesterol Esters	Total Cholesterol, Mg. per 100 Cc.	% Cholesterol Esters	Total Cholesterol, Mg. per 100 Cc.	% Cholesterol Esters
3	106	75	133	74	100	77	1,360	75
5	126	74	...	..	145	74	2,148	64
					185	77	2,040	63
							1,878	60
8	...	..	165	76	118	70	1,040	72
12	126	67	200	71	...	..	1,792	63
16	75	74	153	79	160	71	430	68
17	...	..	...	..	...	..	2,148	67
							1,920	63
							1,902	64
							836	65
21	160	77	190	70	176	76	2,040	68
							1,040	68

TABLE 2.—*Relation of Total Blood Cholesterol to Degree of Vascular Atherosclerosis in Cholesterol-Fed Chicks*

Chick	Total Blood Cholesterol, Mg. per 100 Cc.	Date of Sample	Degree of Vascular Sclerosis	Date of Death
69	1,920	9/15/42	++	1/30/43
	1,046	1/ 6/43		
24	1,360	9/ 1/42	+++	12/ 7/42
	1,040	11/ 6/42		
	430	12/ 1/43		
73	1,878	9/15/42	+++	11/ 3/42
	1,792	11/ 3/42		
59	2,040	9/15/42	++++	1/ 4/43
	836	12/ 8/42		
60	2,148	9/15/42	++++	1/ 2/43
	1,902	12/ 8/42		
67	2,148	12/ 8/42	+++++	1/30/43
	2,040	1/ 6/43		

Blood Cholesterol.—Thirteen blood cholesterol determinations were done on 6 different cholesterol-fed chicks; 5 determinations were done on 4 control chicks; 6 on 4 underfed chicks, and 5 on 2 chicks fed cottonseed oil. The results are presented in table 1. In all determinations in the cholesterol-fed series the total blood cholesterol level was markedly elevated. The cholesterol esters increased proportionately so that the ester percentage remained normal. This differs from our previous observations with younger cockerels receiving larger quantities of cholesterol, in which the percentage of the cholesterol esters was reduced for two to three weeks and then returned to the normal range.[1] This drop in ester percentage was interpreted as evidence of functional damage of the liver. In the older cockerels the liver is evidently able to esterify the cholesterol supplied in a normal degree. As table 2 shows, the total blood cholesterol declined as the chicks grew older, despite continued cholesterol feeding, while in our previous study the levels tended to increase progressively.[1] It appears that older chicks either do not absorb as much cholesterol or else dispose of it more readily than younger chicks.

It is true that younger chicks start out with a higher percentage of cholesterol in their livers[11] and hence may be less able to handle added dietary or exogenous cholesterol. To explain a similar phenomenon in rabbits, Weinhouse[12] advanced the hypothesis that the drop in successive blood cholesterol levels and the loss in weight may result from infiltration of the intestinal mucosa by cholesterol with subsequent impairment of absorption. Cholesterol does become deposited in the intestinal mucosa of the chick, but this occurred in both the young and the older chicks and so would not explain the difference between the two age groups.

We were not able to find any clear correlation between the height of the blood cholesterol level, the duration of cholesterol feeding or the product of the two and the extent of vascular atheroma. Apparently some other variable is concerned in determining the degree of atheroma induced in cholesterol-fed chicks. Marked individual variation in the ability of chicks to assimilate cholesterol has been noted before.[13]

Gross and Histologic Changes Exclusive of the Cardiovascular System.—(*a*) Liver: The liver was grossly abnormal only in the cholesterol-fed chicks. In this group the liver was in each instance enlarged, smooth, greasy and bright yellow-tan. The gallbladder was markedly distended but contained no precipitate or stones. Microscopically, the liver showed small fat vacuoles within the hepatic cells throughout the lobules but most prominently about the central veins. Foam cells were found within small venules in 1 case (fig. 2*A*), similar to the findings of Leary[14] in rabbits. By contrast there was nothing unusual microscopically in the livers of the other groups with the following exceptions: 3 chicks (1 control and 2 fed cottonseed oil) showed moderate fatty metamorphosis; 3 chicks (2 underfed and 1 fed cottonseed oil) had dilated bile canaliculi containing inspissated bile.

(*b*) Spleen: In 2 cockerels in the cholesterol-fed series the spleen was enlarged, and in 6 it was pale pinkish or tan instead of its usual purple color. Microscopically, large pale foam cells were seen, most numerous just beneath the splenic capsule, sometimes occurring in aggregations. These findings were not present in the other groups, in all of which the spleen was normal grossly and microscopically.

(*c*) Kidneys: In the cholesterol-fed chicks the kidneys were grossly somewhat enlarged, yellowish tan and firmer than usual and showed a more granular surface. No microscopic abnormalities were discovered. The kidneys of the chicks not receiving cholesterol were normal grossly and microscopically.

(*d*) Intestine: In the few instances in which the intestine was examined, foam cells were found in the mucosa of the small and the large intestine of cholesterol-fed chicks. The gizzard showed no changes other than vascular.

(*e*) Lungs: The lungs of 3 cholesterol-fed birds showed foam cells within capillaries of the alveolar walls, packed end to end in seeming chains (fig. 2*B*). Foam cells were also seen within the lumens of pulmonary venules (fig. 2*C*). In 2 of these chicks small areas of the lung were solidly packed with foam cells without any accompanying inflammatory reaction. These findings resemble those described by Leary[14] in rabbits.

(*f*) Lymph Nodes: In 3 cholesterol-fed chicks bright yellow enlarged firm mesenteric and femoral lymph nodes were seen. In these nodes, microscopically, the lymphoid tissue was replaced by collections of cholesterol crystals and foam cells and by fibrous tissue.

(*g*) Testes: In all groups except the controls a high incidence of small, undeveloped testes was encountered. The distribution was as follows: 3 of the 10 cockerels in the control group; 8 of the 10 in the underfed group; 10 of the 11 in the group fed cottonseed oil; 8 of the 11 in the cholesterol-fed group. The undeveloped testes were small (1 cm. by 0.5 cm.), firm and yellow, in contrast to the fully developed testes, which measured 3.5 cm. by 1.5 cm. and were whitish, soft and vascular. Microscopically, instead of the glandular appearance with wide seminiferous tubules of the developed testes, there was a solid appearance, with tiny, undeveloped tubules. According to Parker, McKenzie and Kempster,[15] rapid testicular growth occurs in White Leghorns between 8 and 16 weeks of age, so that the cockerels in this study were old enough to have shown development. Kawamura[6a] and Yamaguchi[6b] found testicular atrophy with increased fat content as a result of feeding cholesterol to pigeons. Many factors are known to affect testicular development in birds, such as confinement in cages, quantity of light and quantity of feed,[16] but to our knowledge the inhibitory effect of cottonseed

11. Dam, H.: Biochem. Ztschr. **232**:269, 1931. Entenman, C.; Lorenz, F. W., and Chaikoff, T. L.: J. Biol. Chem. **133**:321, 1940.

12. Weinhouse, S.: Arch. Path. **35**:438, 1943.

13. Sperry, W. M., and Stoyanoff, V. A.: J. Nutrition **9**:157, 1935.

14. Leary, T.: Arch. Path. **32**:507, 1941.

15. Parker, J. E.; McKenzie, F. F., and Kempster, H. L.: Poultry Sc. **21**:35, 1942.

16. Domm, L. V., in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939, chap. 5, p. 227.

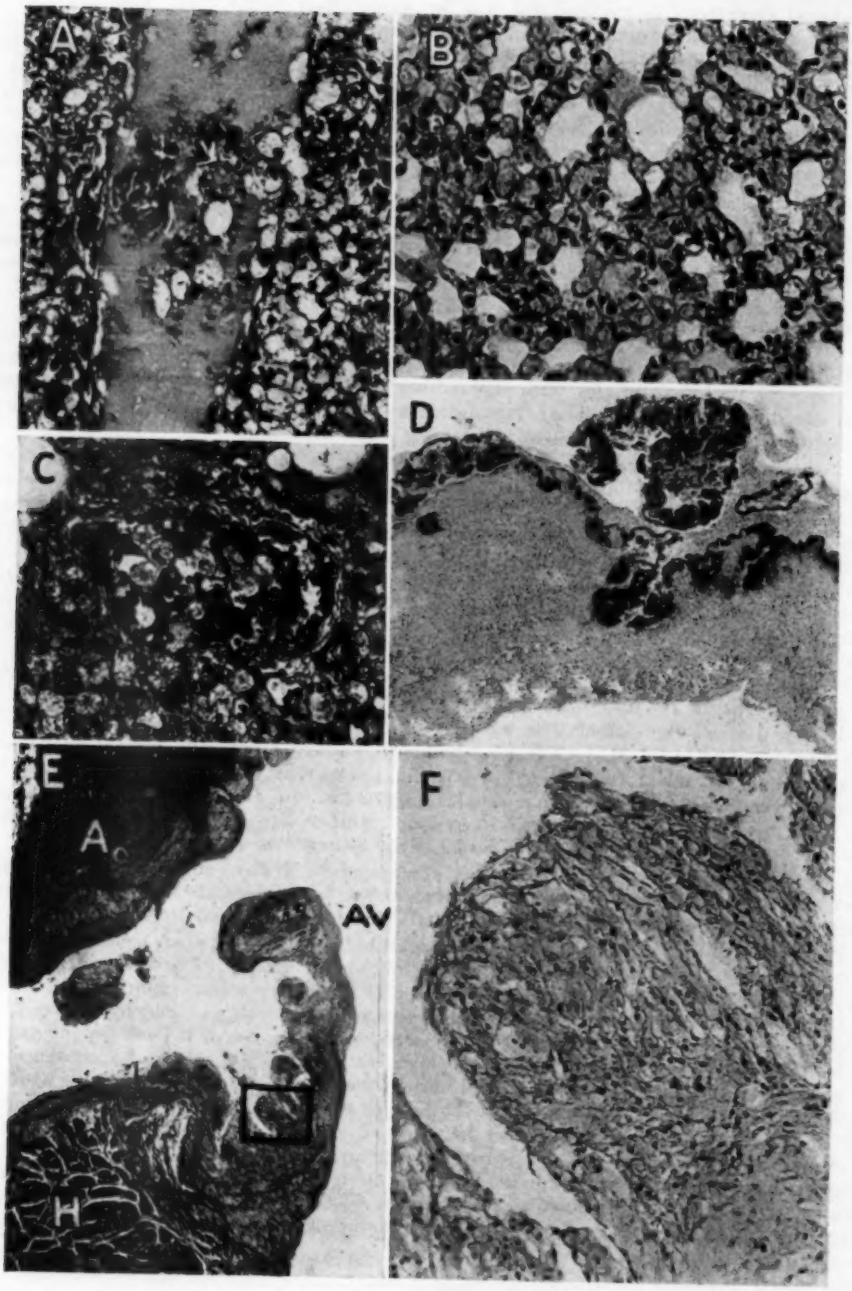

Figure 2

(See legend on opposite page)

oil on the development of the testes has not previously been noted. It may have been due to the associated poor intake of food in the cockerels fed cottonseed oil. Since this sexual underdevelopment occurred frequently in all except the controls, the blood vessel changes occurring only in the cholesterol-fed birds cannot be related to it.

A definite sex difference in the incidence of spontaneous atherosclerosis is consistently found in both animals and man, and this is frequently attributed to more intense vascular stresses in the male and to a more labile cholesterol metabolism in the female. It might therefore be expected that any factor interfering with sexual development of the male animal would tend to inhibit atherosclerosis. Yet such was not the case in our cockerels.

(*h*) Adrenal Glands: The adrenal glands of the cholesterol-fed chicks grossly were whitish yellow rather than the usual deep yellow-tan. The enlargement of the adrenal glands reported in rabbits on a cholesterol diet[17] was not observed in these cholesterol-fed chicks. However, Kawamura[6a] and Yamaguchi[6b] reported enlargement of the adrenal glands following cholesterol feeding in pigeons.

Microscopically, the cortical cells[18] in the cholesterol-fed series appeared swollen and pale, with occasional small vacuoles. However, staining reactions were variable in all groups. Fat stains of frozen sections showed fat in the adrenal glands of all groups, with a slight increase in the amount of fat in the cholesterol-fed birds. Uchiyama[5b] found as much fat in the adrenal glands of control birds as in those of birds fed cholesterol.

(*i*) Pancreas: No gross or microscopic abnormalities were observed.

Gross and Histologic Changes in the Cardiovascular System.—(*a*) Heart: Grossly, the heart showed no changes in most of the chicks except in those in which intercurrent carditis was encountered. This occurred in several chicks in each group (2, 5, 2, 1, in the control, underfed, cottonseed oil–fed and cholesterol-fed groups, respectively), apparently causing death in certain ones that succumbed in the course of the experiment, and was found unexpectedly in others, apparently well, that were killed at the end of the study.[19] The incidence was

17. Wacker, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **74**:416, 1913. Leary.[14]

18. The organization of the adrenal gland of the chick differs from that of man in that columns of cortical and medullary cells are intermingled throughout the organ.

19. This inflammatory carditis remains etiologically unexplained. In many instances it was associated with extensive exudative pericarditis. It appeared as acute or chronic myocarditis, as valvulitis, as endocarditis or as a combination of all. Grossly, the affected hearts were often markedly enlarged. The parietal and visceral pericardia were united by recent thick fibrinous or old, organized fibrous adhesions, so that they could not be separated. Occasionally a pericardial exudate was present in small amount, and the pericardium of one cholesterol-fed bird with pericarditis contained bright yellow deposits. The myocardium was thick and soft with yellowish or purplish areas. In several cases red or purplish vegetations were seen on cusps of the aortic and mitral valves at the line of closure, and in another case a few vegetations extended down onto the endocardium of the left ventricle. Microscopically, the pericardia showed young and old connective tissue and accumulations of inflammatory cells. In most cases there were collections of inflammatory cells in the myocardium, and in an additional case there were definite abscesses and large clumps of bacteria in the myocardium. The vegetations on the valves contained masses of bacteria and fibrin (fig. 2*D*).

Explanation of Figure 2

A, foam cells occurring within a hepatic venule of a cholesterol-fed cockerel. Hematoxylin-eosin; × 320.

B, foam cells within capillaries of the alveolar walls of a lung of a cholesterol-fed bird. Hematoxylin-eosin; × 380.

C, foam cells within a pulmonary venule of a cholesterol-fed cockerel. Hematoxylin-eosin; × 280.

D, section of a vegetation on a cusp of the aortic valve of a chick affected with pancarditis of unknown cause. Note masses of bacteria and fibrin forming a characteristic vegetation of bacterial endocarditis. Hematoxylin-eosin; × 40.

E, atheromatous deposits on a cusp of the aortic valve of a cholesterol-fed chick. Note that the heaped-up deposits are on the aortic aspect of the cusp. *Ao* indicates the aorta; *H*, the heart muscle; *AV*, the aortic valve. Hematoxylin-eosin; × 20.

F, higher magnification of the area marked by the square in *E*, showing the masses of foam cells composing the atheromatous plaques of the aortic valve. Hematoxylin-eosin; × 170.

similar in all groups except the underfed, and the infection played no demonstrable role in the difference in vascular abnormalities observed in the several groups. However, this infection caused an increase in the heart weight/body weight ratios (table 3).

Microscopically, fatty infiltration was seen in 1 or 2 chicks in each group. While the size of the heart muscle fibers varied considerably from chick to chick within each group, no variation between groups could be established.

Atheromatous deposits were found in the aortic and mitral valves in only the cholesterol-fed group. The pulmonic and tricuspid valves were uninvolved. The lesions of the aortic valve consisted of discrete, yellow or white, opaque, pinpoint or heaped-up deposits elevated above the endocardial surface. These were encountered on the aortic surface of the cusps in every cholesterol-fed chick; in 4 birds there were, in addition, similar deposits on the ventricular aspect of the cusps at the line of closure. No valvular rigidity, stenosis, ulceration or calcification was seen. Microscopically, the lesions of the valves were composed of heaped-up subendothelial accumulations of large clear foam cells, with no evidence of an inflammatory process (fig. 2*E* and *F*).

Atheroma of the mitral valve was encountered grossly in 5 cholesterol-fed birds. In all these, elevated discrete pinpoint white lesions involved the ventricular aspect of the aortic

TABLE 3.—*Relation of Heart Weight to Body Weight*

Control Chicks			Chicks Fed Cottonseed Oil			Underfed Chicks			Chicks Fed Cholesterol		
Body Weight, Gm.	Heart Weight, Gm.	Heart Weight Body Weight, %	Body Weight, Gm.	Heart Weight, Gm.	Heart Weight Body Weight, %	Body Weight, Gm.	Heart Weight, Gm.	Heart Weight Body Weight, %	Body Weight, Gm.	Heart Weight, Gm.	Heart Weight Body Weight, %
2,450	18.9	0.77*	936	4.95	0.53	824	3.35	0.40	1,132	7.0	0.62
2,375	11.1	0.47	852	4.85	0.57	680	3.25	0.48	1,263	11.0	0.87
2,026	9.3	0.46	880	6.15	0.70*	594	2.55	0.57	1,188	9.5	0.80
1,788	6.6	0.37	880	3.85	0.44	936	6.25	0.67*	767	3.45	0.45
1,844	8.4	0.46†	1,270	8.85	0.70	678	2.95	0.44	978	5.90	0.60
2,157	10.6	0.49	1,302	7.25	0.53	880	2.9	0.33†	908	4.90	0.55
1,875	6.6	0.35	1,278	5.3	0.41	850	3.0	0.35†	1,020	4.5	0.44
1,984	10.4	0.52*	650	2.35	0.36	590	2.75	0.47	880	3.9	0.44
2,494	....	....	795	5.5	0.69*	1,708	9.75	0.57‡	820	11.8	1.4*
781	3.38	0.43	1,188	6.56	0.55	2,382	10.5	0.44‡	1,334	....	....
.....	....	....	1,076	6.15	0.57	734	....	....	1,788	8.9	0.50
Average obtained exclusive of values marked with an asterisk.....		0.43			0.52			0.45			0.59

* The heart showed myocarditis and pericarditis; hence it was not valid for comparison.
† The heart showed only slight myocarditis microscopically.
‡ This chick was on a full diet the last two months (seventh to ninth month).

leaflet of the mitral valve. They were absent from the free border, and the dotlike lesions often occurred in rows. In 1 instance an additional deposition on the auricular surface of this valve leaflet was found. No gross deformity, ulceration or calcification of the valve resulted. Microscopically, these lesions resembled those of the aortic valve.

(*b*) Thoracic Aorta and Brachiocephalic Arteries (Elastic Arteries): Except in 1 cockerel in the group fed cottonseed oil, no gross or microscopic lesions of the thoracic or elastic aorta were encountered outside the cholesterol-fed group. In the exception a fine tan granularity of the ascending aorta was seen, which microscopically showed slight subendothelial thickening with loose reticular young connective tissue and contained no lipoid (sudan IV stain).

In the cholesterol-fed group the lesions in the thoracic aorta were considerably more extensive than those in the previous cockerels studied by us.[1] Every chick in the group showed extensive atheroma. In the milder cases (6 in number) the lining of the thoracic aorta was deep tannish yellow, granular and without discrete plaques. In the more advanced cases (5 in number) there were irregular knobby plaques and elevations superimposed on the granular deposit. A number of aortas were rigid and grated on cutting, but no gross intimal thrombi or ulcerations were seen. Definite narrowing of the orifices of the brachiocephalic and of the coronary arteries resulted from the presence of the atheromatous plaques.

The distribution of atheroma in the thoracic aorta was fairly consistent. In every case, whether lesions were mild or severe, the sinuses of Valsalva showed considerable involvement, while the region just above was relatively uninvolved. The arch of the aorta and the first part of the descending aorta were again extensively affected, while the involvement tended to

peter out in the lower part of the thoracic aorta, which became relatively smooth and white with isolated raised flat plaques or none at all.

Involvement of the brachiocephalic arteries, which are also elastic arteries, paralleled that of the thoracic aorta in 9 cases. Besides the lesions at the orifices in every cholesterol-fed chick, there was extensive atheroma in the arteries themselves, resembling that in the thoracic aorta. In a tenth cholesterol-fed chick, the brachiocephalic arteries were rigid, were markedly widened and grated on cutting but had a smooth intimal surface. The branches of the brachiocephalic arteries, viz., the brachial and carotid arteries, showed scattered gross yellow elevated atheromatous plaques in every cholesterol-fed chick.

Microscopically, clusters of subendothelial foam cells were found in the aortic lesions as in our earlier study.[1] In addition, the lesions contained fibrous tissue (in some cases hyalinized), fusiform cholesterol clefts, amorphous ground substance ("mucoid degeneration") and granular calcific deposits (fig. 3 *A* and *B*). In 5 cases cartilage, both uncalcified and calcified, was observed in the thoracic aorta and the brachiocephalic arteries. Cartilage plates may occur normally in chicks at the root of the aorta,[5b] but our sections were taken beyond this region. Questionable ulceration of the endothelium was found in 1 case, but in no instance did definite ulceration with thrombosis occur. However, necrosis within the intimal lesions with formation of the so-called "atheromatous abscesses" was frequent (fig. 3 *A*, *B* and *C*). In 5 cases there were wide endothelium-lined channels within the thickened intima, suggestive of the channels or vasa vasorum described by Winternitz, Thomas and Le Compte[20] in human aortas. In 2 instances these were filled with clear foam cells (fig. 3 *D*, *E* and *F*).

The components of the intimal thickening were arranged characteristically in serial layers. Just below the endothelium in 8 instances was a region of foam cells, presumably the most recent addition to the intimal plaque in the vessel wall. In other parts of the same plaque young fibrous tissue occurred just beneath the endothelium; in 3 instances this was hyalinized in its deeper portion. Deeper within the wall were necrotic amorphous zones (mucoid degeneration) containing the fusiform slits of cholesterol crystals (7 cases), often interspersed with fine calcific granules (5 cases). Sometimes there were two distinct cholesterol crystal zones separated by hyalinized connective tissue. In addition, in 5 cases a plaque of cartilage lay just beneath the fibrous or the foam cell layer or just below the cholesterol layer. In few instances was the media found to be invaded by the intimal thickening even though the lesion formed an intima two to three times the thickness of the rest of the vessel wall.

(*c*) Abdominal Aorta: Unlike the thoracic aorta, the abdominal or muscular aorta showed spontaneous lesions in the control, underfed and cottonseed oil–fed chicks. In 3 chicks of each of these groups a lesion, uniform in appearance and location, was encountered. It consisted of an inconspicuous longitudinal firm white ridge which projected into the lumen of the vessel, in some instances making the lumen eccentric. It was located on the anterior aspect of the abdominal aorta, in the interrenal region just above the bifurcation of the aorta. In the aorta of 1 chick fed cottonseed oil a yellow color was seen. This bird had two ridges parallel to each other, one on the anterior and one on the posterior wall. Microscopically, the lesion was encountered in 17 chicks in the groups not fed cholesterol, in addition to the 9 in which it was seen grossly. Thus it was present in 26 of the 32 chicks in these three groups. In a few cases two plaques were found microscopically on cross section, although grossly only one ridge had been seen. The lesion was composed of dense, hyalinized connective tissue (Van Gieson stain) which formed a thick subendothelial plaque in the vessel wall (fig. 4 *A*). Fat was demonstrated by sudan IV staining of frozen sections in 2 cockerels fed cottonseed oil, one of them with the yellow ridge already referred to, as well as in 2 underfed chicks. The fat was small in amount, located in 3 cases deep within fibrous plaques at the junction of intima and media (fig. 4 *A*), and appeared to be intracellular, but no foam cells were present. In 1 underfed chick intracellular fat was seen immediately below the endothelial surface in a region showing only minimal microscopic fibrous thickening. Fat was seen, therefore, in a small early lesion as well as in a late far advanced lesion. In the early lesion the fat was superficial, subendothelial, while in the old lesion it was deep, close to the media. Orcein elastic tissue stain disclosed a well defined internal elastic lamina at the outer boundary of these plaques, with no reduplication of elastic fibers. No cholesterol slits, foam cells or calcific deposits were found in any of these cases. The media was narrowed and compressed under the largest plaques but not invaded. Intimal vasa containing blood cells were discernible in 1 case at the junction of a plaque with the media (fig. 4 *D*).

There is no doubt that this lesion is due to spontaneous intimal fibrosis occurring in the cockerel between the ages of 5 and 9 months. The fibrous character of the lesion is apparent from the start even in the blood vessels with least involvement, and there is nothing to

20. Winternitz, M. C.; Thomas, R. M., and Le Compte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

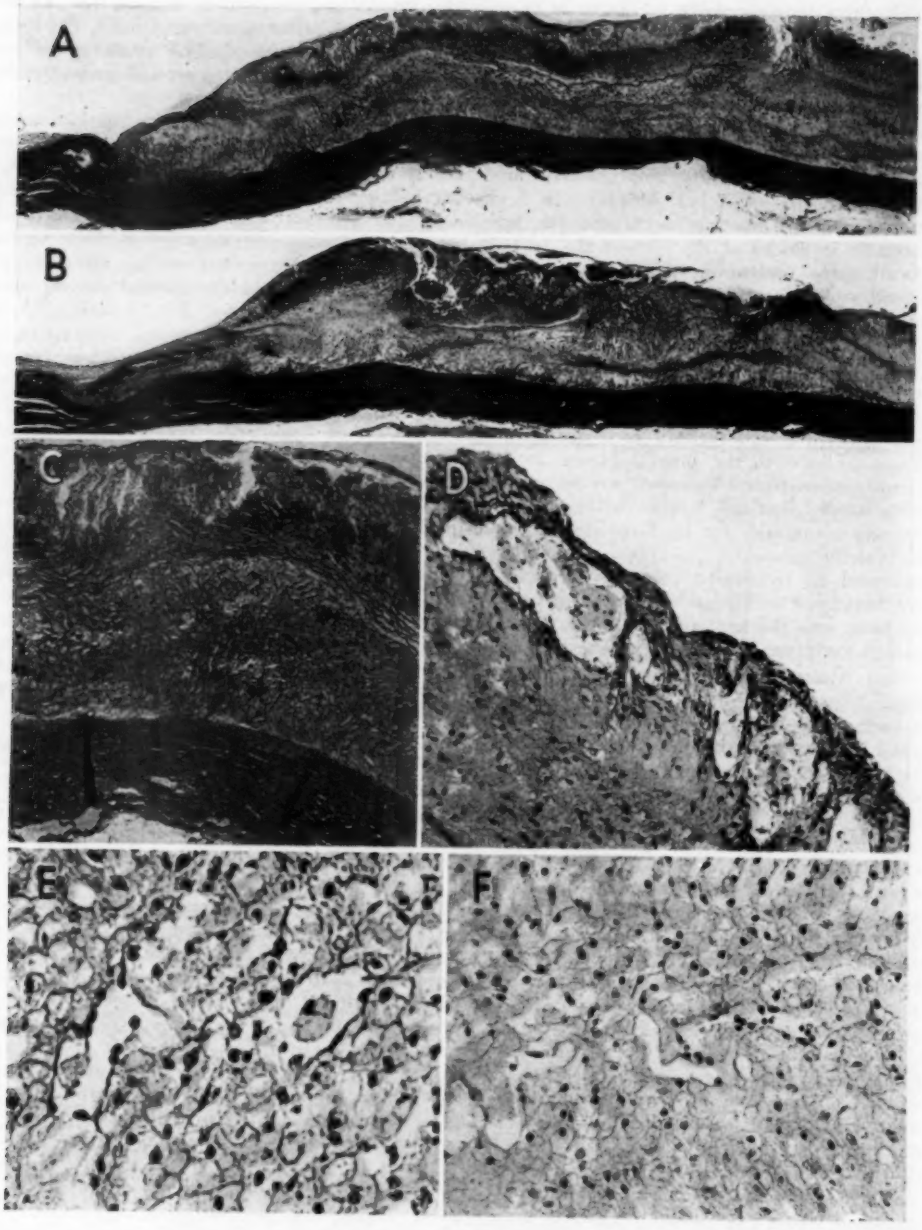

Figure 3

(See legend on opposite page)

suggest that the fibrosis is secondary to atheroma. Even the lesions of this type containing lipoid were unlike those encountered in the abdominal aorta of cholesterol-fed chicks, although the location was identical.

Every cholesterol-fed chick showed gross atheroma on the anterior surface of the abdominal aorta just above the bifurcation, in the interrenal region. The lesion was usually a single, large, bright yellow elevated longitudinal plaque, often double ovoid in shape with overhanging edges and slight depressions at the ovoid centers. Sometimes a fine white ridge similar to that seen in the other groups extended above the yellow plaque. In 6 instances the orifice of one or of each of the common iliac arteries was partly obstructed by extension of the atheroma into the vessel. In 2 birds the entire circumference of the abdominal aorta at this level was affected, with fusiform widening of the vessel's outer diameter and narrowing of the lumen to pinpoint size. The orifices of both renal arteries were narrowed in 1 case.

Microscopically, there was localized subendothelial fibrous and hyaline thickening, similar to the spontaneous fibrosis in chicks not fed cholesterol. In addition, within these fibrous plaques were changes characteristic of atherosclerosis (fig. 4 *B*). In 9 of the 11 cholesterol-fed chicks cholesterol slits were present in the plaques; in 1 there were foam cells; 2 showed necrotic breakdown in the form of so-called "atheromatous abscesses" (fig. 4 *C*); 6 showed calcification.

The arrangement of components in these lesions resembled that in the atheromatous lesions of the thoracic aorta. Closest to the endothelium was young connective tissue or, in the areas of least thickening, foam cells. Hyalinized connective tissue often occurred deeper within the plaque. Below this was a zone of cholesterol clefts in an amorphous ground substance and at the junction of intima and media was an accumulation of calcific granules. In some instances two clearcut zones of cholesterol crystals and calcification were arranged in alternate layers separated by dense connective tissue. In one instance definite zones of hemorrhage were seen within the intima (fig. 4 *E* and *F*). The intima was sometimes ten times the thickness of the remaining vessel wall. There were marked narrowing and partial destruction of the media under the plaque in 7 cases. Complete destruction of the media under the center of the plaque was seen in 2 cases (fig. 4 *E* and *F*). No intimal vasa were seen, but calcium deposits lying within spaces were suggestive of the calcium "liths" within intimal sinusoids described by Winternitz, Thomas and Le Compte.[20] However, no definite endothelium could be seen lining these spaces.

(*d*) Arteries of the Legs: No gross lesions were seen in the common iliac or the femoral arteries in chicks not fed cholesterol. Microscopically, however, 3 chicks in the underfed group and 1 in the group fed cottonseed oil showed thin areas of subendothelial proliferation of fibrous tissue (fig. 4 *G*). There was no development of new muscle cells as reported by Yamagiwa and Adachi,[21] nor any reduplication of elastic elements.

21. Yamagiwa, K., and Adachi, O.: Verhandl. d. japan. path. Gesellsch. **4**:55, 1914.

Explanation of Figure 3

A, atherosclerosis of the thoracic aorta of a cholesterol-fed chick (longitudinal section). Note the large calcific plaque at the left end of the section and the fine calcific granules scattered throughout. Just below the endothelium a zone of necrosis can be recognized. Below this are calcific granules and tiny cholesterol clefts. Next is a band of wavy connective tissue. Below this, more calcium deposits and cholesterol clefts are recognized. The sclerotic plaque is about three times the thickness of the remaining aortic wall. Hematoxylin-eosin; × 18.

B, atherosclerotic plaque of the thoracic aorta (longitudinal section) of a cholesterol-fed chick. Note the successive zones composing the plaque. Orcein elastic stain shows an abundance of elastic tissue in the media and its absence in the plaque. × 18.

C, higher magnification of a portion of the thoracic aorta shown in *A* illustrating the elements of the plaque. Hematoxylin-eosin; × 44.

D, foam cells lying within endothelium-lined channels in the thoracic aorta of a cholesterol-fed chick (longitudinal section). Hematoxylin-eosin; × 170.

E, endothelium-lined channels within a foam cell plaque in the thoracic aorta of a cholesterol-fed chick. Note the single foam cell lying within one of these channels. Hematoxylin-eosin; × 400.

F, vasa vasorum within a foam cell plaque in the thoracic aorta of a cholesterol-fed chick. Nucleated red blood cells can be seen lying within the vas. Hematoxylin-eosin; × 300.

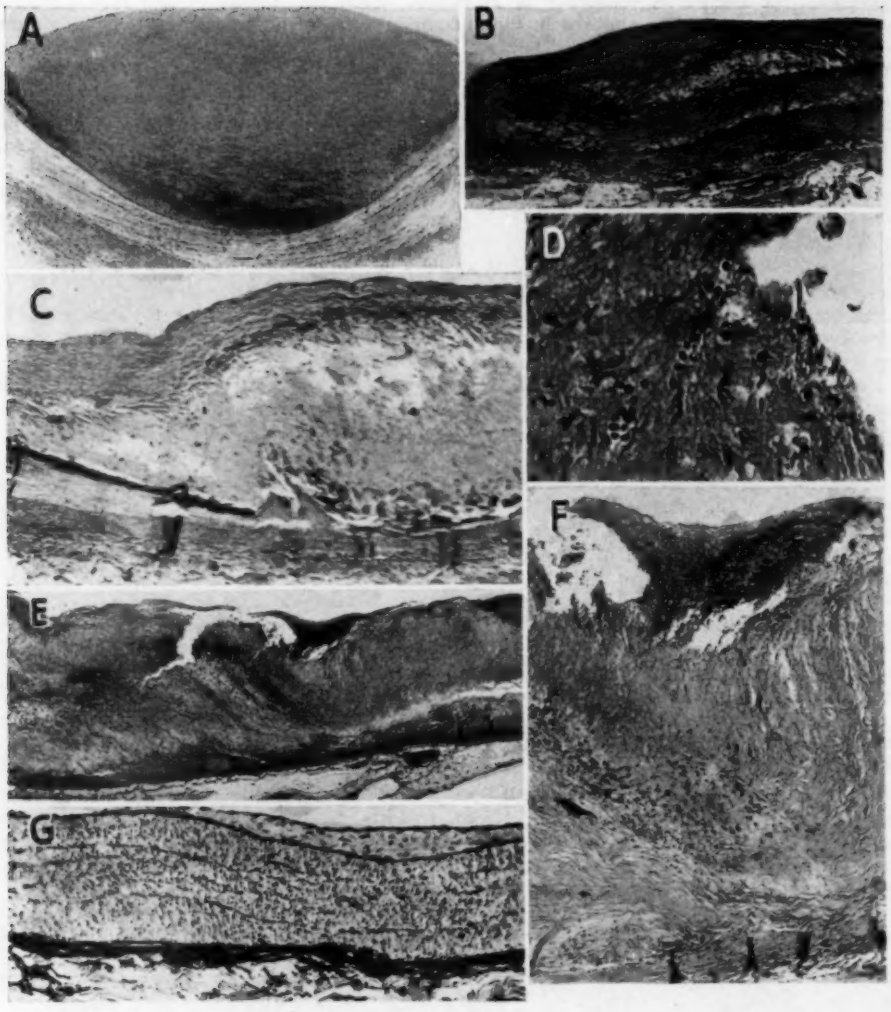

Fig. 4.—*A*, fibrous intimal plaque in the abdominal aorta of a chick fed cottonseed oil (transverse section). Note lipid in the plaque near the media. Frozen section; sudan IV; × 24.

B, fibrous and atheromatous plaque in the abdominal aorta of a cholesterol-fed cockerel (transverse section). Note the area of mucoid degeneration containing cholesterol slits deep within the plaque, adjacent to the media. Hematoxylin-eosin; × 34.

C, "atheromatous abscess" in the abdominal aorta of a cholesterol-fed cockerel (transverse section). Note the overlying fibrosis and the dark thin calcific plaque on the left next to the media. Hematoxylin-eosin; × 32.

D, vasa vasorum containing nucleated red blood cells in wall of the abdominal aorta of a chick fed cottonseed oil (transverse section). These vasa lie at the junction of a fibrous intimal plaque with the media. Hematoxylin-eosin; × 360.

E, atherosclerosis of the abdominal aorta of a cholesterol-fed chick. Note the dark area of hemorrhage just beneath the endothelium. Calcific granules are also seen. The media is almost entirely destroyed. Hematoxylin-eosin; × 13.

F, higher magnification of a portion of the plaque shown in *D*. Note the hemorrhage at the top. Below it are "mucoid degeneration," cholesterol clefts, calcific granules and fibrosis. Hematoxylin-eosin; × 44.

G, thin fibrous intimal plaque in the femoral artery of a chick fed cottonseed oil (longitudinal section). Orcein elastic stain; × 150.

In 9 cholesterol-fed chicks one or both common iliac arteries showed grossly elevated yellow intimal plaques which narrowed the lumen. In 1 cockerel a large saccular aneurysm filled with a laminated thrombus (fig. 5) was found involving 1 cm. of the right iliac artery. Microscopically, beneath the thrombus of this aneurysm was amorphous eosinophilic material containing cholesterol crystals and foam cells. This merged into a zone of dense hyaline connective tissue interspersed with calcific granules and cholesterol slits. The media of the vessel was entirely destroyed.

Seven of the cholesterol-fed chicks also showed gross lesions in the femoral arteries, which in the earlier stages consisted of fine linear transverse whitish streaks and pinpoint dots. Later, definite small white or yellow elevated plaques developed about the orifices of

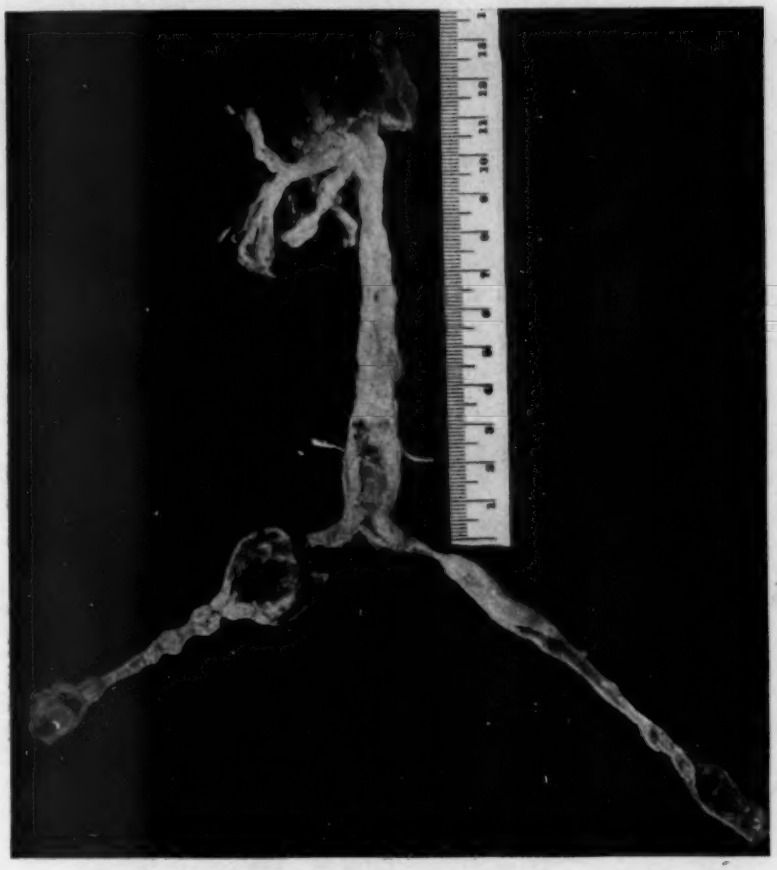

Fig. 5.—Saccular aneurysm of the left iliac artery and aneurysms of both femoral arteries in a cholesterol-fed chick. Note the very narrow portion of the right iliac artery illustrating the normal caliber of this vessel. Below this is an unopened portion of the right iliac artery in which no gross lumen could be found. Note beadlike widened areas of the left femoral artery below the large aneurysm. Note also the fusiform widening of the abdominal aorta and the thick plaque in it. The heart and the brachiocephalic arteries are attached to the aorta.

branching vessels, resulting in considerable narrowing. In the larger deposits there were localized bulgings so that the unopened vessel resembled a string of beads. Sometimes this beading was found as far down in the leg as the vessel could be followed, and in 1 bird there were multiple aneurysms of the femoral artery (fig. 5). In transverse sections, these saccular dilatations occurred at the sites of branching, and the lumens were almost completely occluded. Microscopically, the lesions were composed of localized heaped-up accumulations of young connective tissue cells and foam cells. The aneurysmal dilatations microscopically showed multiple eccentric minute lumens, evidently channels of recanalization, while the original

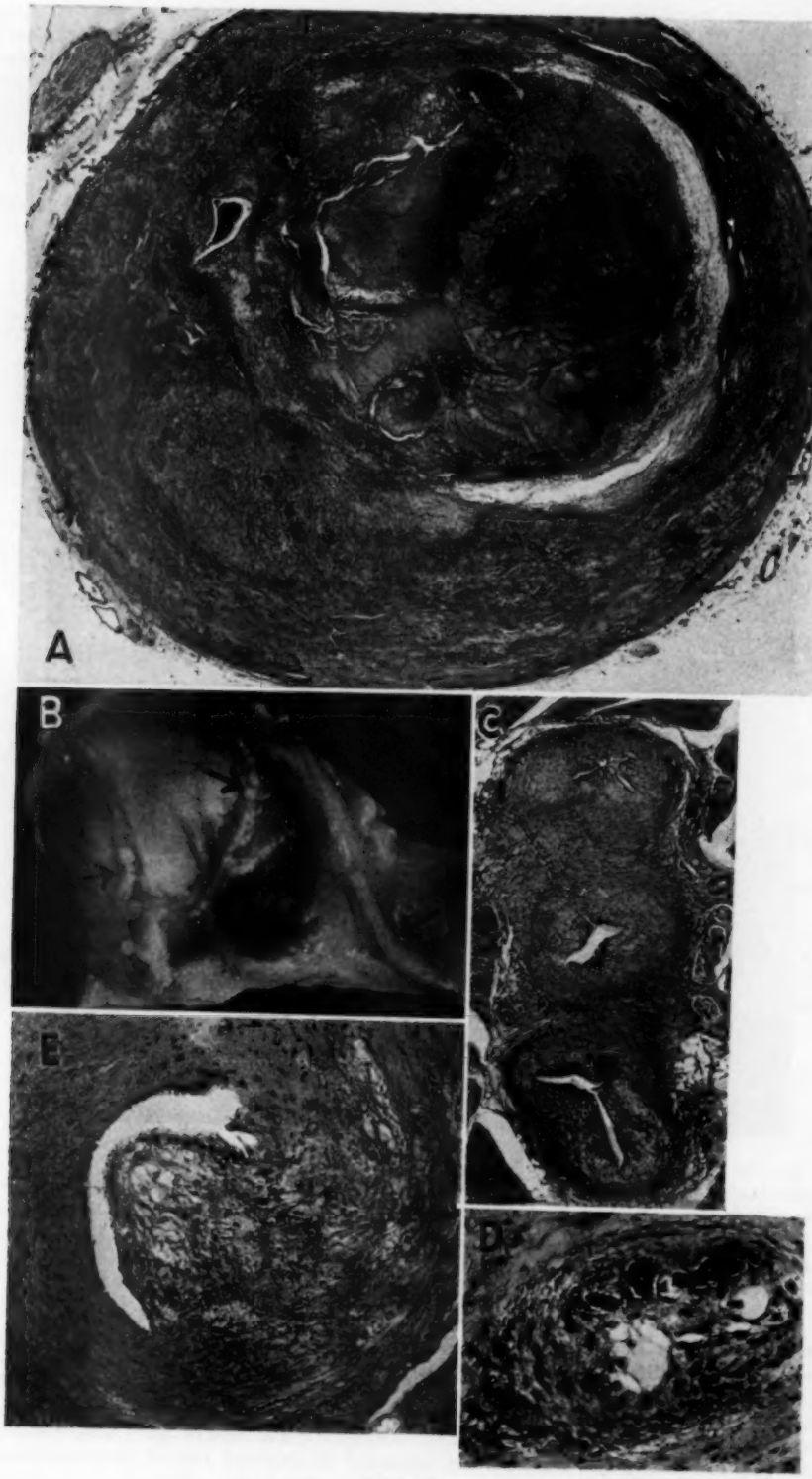

Figure 6
(See legend on opposite page)

dilated lumen was filled with thrombotic, necrotic material, giant cells containing cholesterol crystals, connective tissue, calcium "liths" and tiny collections of calcific granules (fig. 6*A*).

(*e*) Gastric and Mesenteric Arteries: The cholesterol-fed chicks showed gross lesions in the gastric and mesenteric arteries similar to those in the femoral arteries, including the beadlike dilatations (fig. 6*B*). Microscopically also, the lesions resembled the lesions of the femoral arteries (fig. 6 *C*, *D* and *E*; fig. 7*A*). However, some of the microscopic intestinal arteries showed areas of medial calcification (fig. 6*D*) and in one artery foam cells were recognized in the outer part of the media adjacent to the adventitia, possibly at a point of branching (fig. 6*E*). A small artery showing calcium deposits seemingly in sinusoids of its wall as described by Winternitz, Thomas and Le Compte [20] is shown in figure 6*D*. No lesions were encountered in these arteries in the chicks not fed cholesterol.

(*f*) Coronary Arteries: Atheromatous plaques narrowing the ostiums of the coronary arteries affected 8 cholesterol-fed chicks. The ostium of the right coronary artery alone was affected in 1 case; the ostium of the left coronary artery alone, in 3 cases, and the ostiums of both arteries, in 4 cases.

Gross atheroma of the coronary arteries was encountered in only 3 cholesterol-fed chicks. Gross yellow white plaques were seen in the first few millimeters of the right coronary artery as it descended deep within the muscle of the septum. Microscopic atheroma of the coronary arteries was seen in 2 additional birds. In 1 chick two microscopic coronary arteries were almost completely replaced by amorphous material, leaving only minute lumens (fig. 7*B*). In another bird the involved coronary artery showed a large intimal calcified plaque with overlying foam cells, leading to narrowing of the vessel lumen (fig. 7*C*). No myocardial fibrosis or degeneration was found accompanying these lesions of the coronary arteries. There was no striking difference in the heart weight/body weight ratio between the cholesterol-fed chicks and those chicks not fed cholesterol in this series (table 3) such as was found in the rabbits studied in this laboratory [10] and was suggested by the data concerning our previous series of chicks.[1] This may depend on the paucity of extensive lesions of the coronary arteries in the present series. The encroachment on the mouths of the coronary vessels described apparently was not sufficient in degree to cause an increase in relative heart weight. In a number of instances a striking increase in heart weight was associated with the inflammatory carditis, and this was apparently similar in extent in the four groups studied.

(*g*) Other Small Visceral Arteries: Despite extensive microscopic examination, few changes were seen in the small visceral arteries in chicks not fed cholesterol. In only 2 chicks were lesions found, viz., a slight intimal thickening composed of young connective tissue in a large microscopic splenic artery of a cockerel of the control group and a definite subendothelial fibrous plaque in a large microscopic ovarian artery in the hen inadvertently included in the underfed group.

On the other hand, atheroma of the small arteries of the spleen was common in the cholesterol-fed chicks. The infiltration of these splenic arteries was so extreme that they appeared as empty spaces or "holes" in the usual paraffin sections, giving the spleen a Swiss cheese appearance. In many sections arterial walls were scarcely discernible as such. The entire intima and media seemed to have been replaced by lipoid, which had dissolved out in the process of preparing the sections. Other, larger splenic arteries were almost completely

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EXPLANATION OF FIGURE 6

A, atherosclerotic obliteration of the femoral artery of a cholesterol-fed chick. Note the marked fibrous thickening of the wall, the large mass of thrombotic material at the right center and the small lumen at the left. Other minute lumens can be seen. The media is entirely destroyed, and the vessel is many times its normal caliber. Tiny cholesterol clefts are abundant. Iron-hematoxylin; × 15.5.

B, tortuosity and beading of the medium-sized arteries of the gizzard due to atherosclerosis. Arrows indicate three widened arteries on the surface of the gizzard of this cholesterol-fed chick.

C, atherosclerotic thickening at the point of branching of a small tortuous artery in the wall of the gizzard of a cholesterol-fed chick. Note the narrowing of the lumen. Hematoxylin-eosin; × 44.

D, calcium "liths" lying within apparent channels in the wall of a small artery of the gizzard of a cholesterol-fed chick. Hematoxylin-eosin; × 260.

E, foam cells in an elevated plaque and also at the outer border of the media in a small artery of the gizzard of a cholesterol-fed chick. This plaque appears to be at a point of branching. Hematoxylin-eosin; × 140.

obliterated by amorphous basophilic material containing cholesterol crystals. In these arteries one or more slitlike lumens, probably channels of recanalization, were seen (fig. 7 *D*).

(*h*) Pulmonary Arteries: No gross or microscopic lesions of the pulmonary arteries were seen in chicks, whether cholesterol fed or not. This is in striking contrast to the findings in rabbits, in which cholesterol feeding usually results in gross atheroma of the pulmonary arteries.[14]

(*i*) Veins: The veins were not examined extensively grossly. Only two gross lesions were encountered, both in the femoral veins of cholesterol-fed chicks. In both cases definite elevated yellow atheromatous plaques were seen, microscopically consisting of localized sub-

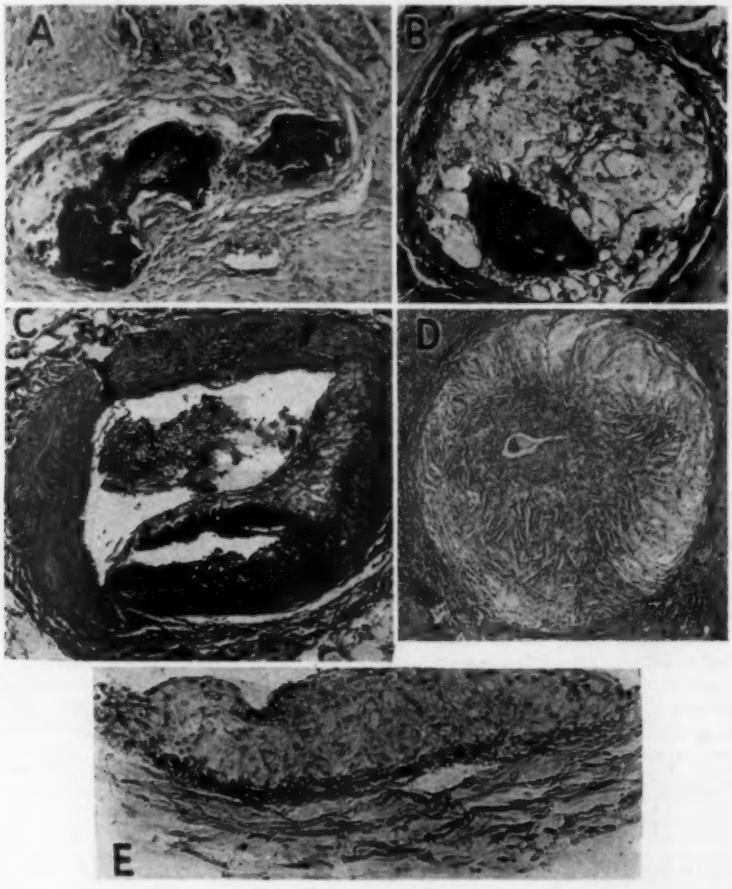

Fig. 7.—*A*, obliteration by a calcific mass of a small artery of the gizzard of a cholsterol-fed chick. Hematoxylin-eosin; × 140.

B, obliteration of a small coronary artery by an accumulation of foam cells and a calcific plaque in a cholesterol-fed chick. A tiny lumen is barely discernible at the center. The media is narrowed. Hematoxylin-eosin; × 140.

C, intimal calcific plaque and overlying atheroma in the coronary artery of a cholesterol-fed chick. Note invasion of the media by the calcific plaque. Hematoxylin-eosin; × 100.

D, atherosclerotic obliteration of a splenic artery of a cholesterol-fed chick. Note "mucoid degeneration" and large cholesterol clefts, as well as fibrous tissue. Hematoxylin-eosin; × 40.

E, foam cells forming an atheromatous plaque in the femoral vein of a cholesterol-fed bird (longitudinal section). Hematoxylin-eosin; × 170.

endothelial accumulations of foam cells (fig. 7 *E*). One of these was associated with enlargement of femoral lymph nodes.

COMMENT

The literature on experimental arteriosclerosis is replete with statements that atherosclerosis cannot be induced in omnivores or carnivores by cholesterol feeding and that cholesterol causes lesions in the herbivorous rabbit (and guinea pig) because it is foreign to the rabbit's usual metabolism.[22] Our earlier experiments [1] clearly showed that feeding cholesterol in cottonseed oil will cause atheroma in the omnivorous chick and that the blood cholesterol of an omnivore can be elevated by cholesterol feeding. Our present results show more specifically that cholesterol itself, and not cottonseed oil or underfeeding, is responsible for the production of vascular lesions in the chick. Furthermore, not only can simple atheroma be thus induced, but also all the stages of the lesions commonly designated as atherosclerosis, namely, intimal lipoidosis, fibrosis, hyalinization, calcification, ulceration and aneurysm formation. In the light of our results it seems that too much emphasis has been placed on the herbivorous character of the rabbit in evaluating the rabbit experiments.

While cottonseed oil feeding and underfeeding may be contributing factors in the development of atherosclerosis, cottonseed oil feeding alone in the chick as in the rabbit [22a] does not result in vascular lesions. Similarly, generalized undernutrition through underfeeding of a balanced mash, without any specific deficiencies, will not of itself cause atheroma, although undernutrition has been related both to the development of fatty liver and the elevation of blood cholesterol. Spontaneous arteriosclerosis has a clearcut higher incidence in male than in female chicks, but the testicular underdevelopment occurring in cholesterol-fed cockerels did not prevent atheromatosis. Apparently, therefore, absence of the hormones elaborated by the mature testis does not interfere with the atheromatosis induced by cholesterol feeding. Our experiments, however, do not reveal whether or not the administration of estrogen to these eunuchoid cocks would have an inhibitory effect on the vascular lesions. Experiments on rabbits indicate that both estrogens and androgens prevent cholesterol atheromatosis in females but not in males.[23]

An objection frequently raised to the experiments on rabbits is that the lesions caused by cholesterol feeding, while they resemble those of man, are totally unlike the vascular changes occurring spontaneously in rabbits.[24] In chicks as in rabbits the human arterial lesions have been clearly reproduced. But unlike the cholesterol-induced lesions in rabbits, those in chicks do resemble the spontaneous lesions. Fox [3] described the characteristic spontaneous arteriosclerosis of birds as consisting of lumps and streaks of yellow atheromatous material, most prominent in the sinus of Valsalva and the arch of the aorta. He found thinning and distortion of vessel walls with narrowing of the lumens to slitlike apertures. In thin-walled vessels the yellow deposits may be visible externally. This description is so applicable that it might have been written for the lesions induced by cholesterol in our chicks. Fox found such atheromatous deposits in 5 of 100 ground fowl averaging 3 years of age. From our observation and that of others [25] there is another spontaneous lesion. It is intimal fibrosis, and it occurs in much higher incidence in the muscular arteries of younger cockerels (26 of 32). While it may contain lipoid, it is clearly different from the lesion described by Fox. We must conclude that there are two distinct

22. (*a*) Duff, G. L.: Arch. Path. **20**:81 and 259, 1935. (*b*) Hueper, W. C.: Medicine **20**:397, 1941. (*c*) Weinhouse.[12]

23. Ludden, J. B.; Bruger, M., and Wright, I. S.: Arch. Path. **33**:58, 1942.

24. (*a*) Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 10, p. 271. (*b*) Duff.[22a]

25. Kesten and others.[5a] Uchiyama.[5b]

types of spontaneous intimal arterial change in fowl and that by cholesterol feeding we have been able to reproduce the one which occurs at the more advanced age.

Independent contemporary investigators have advanced several apparently unrelated and conflicting hypotheses to explain the genesis of intimal atherosclerosis, as a result of their particular observations. Leary [26] expressed the belief that cholesterol-laden cells are liberated into the blood stream from the liver after cholesterol feeding, from the brain after injury or from areas of infectious exudate. These cells traverse the pulmonary circuit to enter the systemic arterial tree, where they actively penetrate the endothelium and accumulate to initiate the formation of atheromatous plaques. Localization in arteries is ascribed to "chemotaxis," and the precise distribution within a given artery is attributed to local mechanical stresses.

On the other hand, Hueper [27] concluded from his observations that macromolecular colloids (carbohydratic or lipoidal) form films on the arterial lining, interfering thereby with the nutrition of the vessel wall. This in turn stimulates swelling and proliferation of the endothelial lining cells, which take up the colloid, becoming transformed into foam cells. In this manner the endothelial cells form plaques and may encroach on the inner media. He acknowledged having seen vacuolated mononuclear cells in blood smears but expressed the opinion that these do not participate in producing atheroma. This view is quite contrary to that of Leary [14] and Anitschkow,[24a] among others, who maintained that the vascular endothelial lining cells over an atheromatous plaque remain intact and possess no phagocytic power. Hueper attributed the localization of lesions to static mechanical forces brought about by posture and to nutritive inadequacies resulting from local excessive demand for blood. Importance is also accorded changes in flow within arterial vasa occurring through hypertonus or hypotonus of the vessel wall.

A third view has recently been advanced by Winternitz, Thomas and Le Compte,[20] who stated that the coincidence of intimal vasa vasorum, intimal hemorrhage and intimal sclerosis was too frequent to be without importance. They demonstrated arterial intimal vasa, not ordinarily seen, which originate at the vicinity of the orifices of branching vessels in healthy adult human arteries. Such intimal vasa are also abundantly found in the human pulmonary arteries and in veins. In arteries showing atheromatosis the intimal vasa vasorum are found in great abundance about every intimal plaque. Winternitz and associates expressed the belief that hemorrhages or exudates from these vasa (from nutritional, chemical, mechanical or infectious causes) may lead to intimal necrosis with cell debris containing lipoid and thus produce atheroma. They also demonstrated the formation of calcified masses or liths within stagnant intimal sinusoids. Hueper [27a] has suggested that some plaques may arise by transformation of the endothelial cells lining such vasa vasorum as well as those of the main vascular lining into foam cells.

Our observations on cholesterol atherosclerosis confirm some of the observations of these workers. We have seen foam cells in hepatic venules (fig. 2 *A*) and in pulmonary capillaries (fig. 2 *B*) and venules (fig. 2 *C*). We have observed intimal vasa in the thoracic and the abdominal aorta (fig. 3 *D*, *E* and *F*). In addition, we have found something hitherto not described, namely, foam cells within these vasa (fig. 3 *D*, *E*, *F* and 4 *D*). We have, of course, found the whole gamut of changes generally recognized to occur in atherosclerosis with clear indication that foam cells are the initiating factor. These foam cells may be migratory cells arriving via the blood stream from sites of injury, inflammation or metabolic overload. They may penetrate the endothelial lining, particularly when others of

26. Leary, T.: Arch. Path. **17**:453, 1934; **21**:459, 1936; footnote 14.
27. (*a*) Hueper, W. C. : Arch. Path. **34**:883, 1942; footnote 22 *b*.

them have blocked vasa vasorum and altered the nutrition of the intima. Coating of the intima by cholesterol films may play a part in further altering the nutrition of the intima when blood levels are markedly elevated through feeding experiments, but it seems unlikely that "coating" operates in spontaneous atherosclerosis. There is no reason to believe that foam cells are of a single origin. The liver, the brain and infectious exudates have been suggested as probable sources by Leary.[14] It is possible that when the intimal nutrition is impaired the endothelial cells, ordinarily not phagocytic, become so and by proliferation contribute to the formation of plaques.

Regardless of the possible origins of foam cells, we are inclined to believe, as a working hypothesis, that migratory, blood-borne foam cells enter the orifices of the intimal vasa vasorum by virtue of the peculiarities of flow at points of branching, obstruct these vessels and start the series of events—necrosis, deposition of cholesterol, fibrosis and calcification—which characterize atherosclerosis. Contributing to this process is the strain to which the vascular regions of the "sites of predilection" are subjected, since they tend to be those localities where eddying and turbulence are at a maximum. Thus the sinuses of Valsalva, the aortic arch, the bifurcation of the abdominal aorta, the points of branching of arteries and the surfaces of the mitral and aortic valves facing the pools of eddying blood are the sites of atheroma. Contributing to the entrance of these large foam cells into the orifices of intimal vasa may be their tendency to move slowly in the peripheral portion of the blood stream and to stick to the lining wall. They would also be more likely to sediment out in regions where flow is slower because of widening of the bed and local low pressures created by eddies. Once a plaque develops, extension may be facilitated by (1) the deformation of the vessel wall, causing adjacent areas of turbulent flow, (2) the impairment of nutrition of adjacent areas due to interruption of flow through the vasa, (3) the irritant effect of the liberated cholesterol and finally (4) the formation of new vasa in a portion of vessel not previously rich in intimal vasa. These preformed or new-formed vasa may subsequently become obstructed by foam cells, initiating new lesions or extensions of the old.

An apparent contradiction to the foregoing working hypothesis is the absence of lesions of the pulmonary artery in the cholesterol-fed chick (as also in most cases in man) and their presence in the rabbit. While it might indicate the absence of vasa vasorum in the pulmonary artery of the chick, this would hardly account for the scarcity of lesions in man in whom the pulmonary artery and its branches are especially rich in intimal vasa. This contradiction may be due to a smoother flow of blood in the lungs of the chick (and man) than in the lungs of the rabbit, and may be related to a difference in posture.

Another apparent contradiction is the occurrence of atheroma in the veins of the legs in some chicks. Turbulence of flow may, however, occur in veins, especially where enlargement of adjacent lymph nodes has caused compression. Intimal vasa are abundant in human veins and may be in the veins of the chick, also. Furthermore, in birds as in man the heart is located at a relatively higher level than in the quadruped rabbit, so that the veins of the legs are subjected to a greater gravitational distending force, which may be contributory.

It is apparent from the foregoing comment that our present view as to the genesis of experimental atherosclerosis is a combination of features previously suggested, in which plugging of intimal vasa vasorum by foam cells is the keystone. We are inclined to view spontaneous atherosclerosis as of similar origin, resulting not from excess cholesterol in the diet but rather from disturbances in the trans-

portation of cholesterol in the body. In other words, experimental cholesterol feeding accelerates a process which presumably occurs intermittently and slowly in the development of spontaneous atherosclerosis. The presence of cholesterol in the spontaneous lesions and the ability to induce lesions by cholesterol feeding in an omnivore as well as in herbivores point to the etiologic importance of cholesterol itself. It would seem, although it has not been proved as yet, that the spontaneous as well as the induced lesions are fundamentally cholesterol engendered. The selection of certain localities depends on (1) the blood pressure in the vessel, (2) the turbulence of flow, acting as a mechanical irritant, and (3) the presence of intimal vasa vasorum.

SUMMARY AND CONCLUSIONS

Forty-three 3 month old cockerels were divided into four groups. One group received liberal amounts of an adequate diet for six months. A second group received the same diet plus cottonseed oil. The third group was placed on restricted feeding and the fourth group received 2 per cent cholesterol in cottonseed oil, added to the basic mash. Every cholesterol-fed chick acquired atherosclerosis of the thoracic and the abdominal aorta and of the major branches. Atherosclerosis of the splenic and the coronary arteries likewise resulted. The lesions showed accumulation of lipoid-containing foam cells, fibrosis, deposition of cholesterol crystals, calcification and cartilage formation. No foam cell atheroma or calcification developed in any chick not fed cholesterol, but the cockerels not fed cholesterol showed a high incidence of intimal fibrosis of the abdominal aorta with or without lipoid.

It is therefore concluded that: 1. Atherosclerosis with intimal thickening by accumulation of foam cells, fibrosis, calcification and deposition of cholesterol can be produced in the omnivorous chick by cholesterol feeding. 2. Feeding of cottonseed oil alone does not produce atheroma. 3. Simple underfeeding will not cause vascular atheroma. 4. The earliest spontaneous vascular lesion in the White Leghorn cockerel is intimal fibrosis of the abdominal aorta with and without lipoid. 5. Foam cells enter the intimal vasa vasorum of the thoracic aorta in cholesterol-fed chicks. 6. The working hypothesis advanced for the genesis of these lesions is applicable also to spontaneous atherosclerosis.

STUDIES OF NORMAL AND ABNORMAL MITOTIC ACTIVITY

III. THE RATE AND PERIODICITY OF MITOTIC ACTIVITY IN REGENERATING EPIDERMIS OF HEALING WOUNDS IN RABBITS

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CLEVELAND

The purpose of this study is to delineate further the character of neoplastic growth. The term "growth" is used herein to mean increase in number of cells by mitosis. Attention is focused on the rate and diurnal periodicity of mitotic activity for the following reasons:

1. The only characteristic common to all tumors is autonomous growth, and the chief, if not the only, means of growth is mitosis.

2. All studies of rate and periodicity of mitotic activity in normal animal tissues reported thus far [1] with a single exception [2] have shown a diurnal variation in the rate of cell division. These features (when and how often mitosis occurs) are ultimate expressions of controlled growth.

3. A study of three different normal tissues of the albino rat, renal cortex, epidermis and submaxillary salivary gland, obtained simultaneously from the same animals, showed a different curve of diurnal rate of mitosis for each. This was interpreted as indicating that the factors responsible for diurnal fall and rise in mitotic activity do not act on the body as a unit, for then each of the three tissues would have had a similar curve; they do not act on the cell as a unit, for then there would be no curve; but they act on or in the organ as a unit.[11]

4. In experimental epidermoid carcinoma of young male CBA mice the average rate of mitotic activity for a twenty-four hour period was one and eight-tenths times that in normal epidermis from the same animals; but the carcinoma, unlike the normal epidermis, showed no diurnal periodicity of mitotic activity. The rate of growth in the tumors remained at the same level throughout the twenty-four hour period and equaled but did not exceed the maximum rate of growth in the normal epidermis. The principal difference between the controlled growth in the normal epidermis and the uncontrolled or autonomous growth in the carcinoma was the absence in the neoplasm of a diurnal period of decreased mitotic activity.[3]

Whether or not this difference in mode of growth is characteristic of neoplasm will be determined not only by observations on other tumors in various species of animals at different ages but also by studies of mitotic activity in conditions of non-neoplastic accelerated growth.

From the Institute of Pathology, Western Reserve University.

1. (*a*) Fortuyn–van Leyden, C. E. D.: Verhandl. d. k. Akad. v. Wetensch. **19**:38, 1917; **29**:979, 1926. (*b*) Ortiz Picón, J. M.: Ztschr. f. Zellforsch. u. mikr. Anat. **23**:779, 1933. (c) Carleton, A.: J. Anat. **68**:251, 1934. (*d*) Cooper, Z. K., and Schiff, A.: Proc. Soc. Exper. Biol. & Med. **39**:323, 1938. (*e*) Blumenfeld, C. M.: Anat. Rec. **72**:435, 1938; Science **90**:446, 1939. (*f*) Cooper, Z. K.: J. Invest. Dermat. **2**:289, 1939. (*g*) Broders, A. C., and Dublin, W. B.: Proc. Staff Meet., Mayo Clin. **14**:423, 1939. (*h*) Cooper, Z. K., and Franklin, H. C.: Anat. Rec. **78**:1, 1940. (*i*) Blumenfeld, C. M.: Arch. Path. **33**:770, 1942.

2. Elliott, H. C.: Am. J. Anat. **58**:127, 1936.

3. Blumenfeld, C. M.: Arch. Path. **35**:667, 1943.

This paper is a report of a study of the diurnal rate and periodicity of mitotic activity in the regenerating epidermis of wounds of the skin of the rabbit, selected as an example of non-neoplastic accelerated growth.

MATERIALS AND METHODS

Six wounds were made in the dorsal skin of each of 12 young male albino rabbits. Two rabbits died before the experiment was completed. Three wounds were placed on each side of the vertebral column in the cervicothoracic, thoracolumbar and sacral regions. In each rabbit one wound was made every four hours, beginning at 12 noon. To obviate the possible effects of regional differences in mitotic activity, the initial wound was made at a different site in each 6 animals, and subsequent wounds were placed in clockwise rotation.

A wound was made by cutting off most of the hair with scissors, raising a fold of skin with forceps and cutting off a full thickness piece of skin. The maximum diameter of the oval wounds thus made ranged from 5 to 12 mm., a majority being 8 mm. No anesthetic, antiseptic, shaving, depilatory or dressing was used because of the possible effect on mitotic activity. The piece of skin cut off was immediately fixed in Bouin's fluid, to serve as the control specimen. Exactly seven days later the wounds were cut off at the same hour and in the same order and manner in which they were made, and similarly fixed. An interval of seven days was selected because Spain,[4] Spain and Loeb,[5] Akaiwa[6] and Arey[7] had observed maximum mitotic activity in healing wounds five to seven days after they were made.

The control and wound specimens were sectioned perpendicularly to the surface at 8 microns and stained with Harris' hematoxylin and eosin. Mitotic activity in each control specimen was determined by counting the mitoses identified at a magnification of 900 diameters in the epidermis of six sections 32 or more microns apart. To obtain a unit for comparison of individual values of mitotic activity, the volume of the epidermis in which the mitoses were counted was determined as follows: The image of the epidermis magnified three hundred and twenty times by an Edinger projection microscope was outlined with a planimeter (Keuffel and Esser no. 4230). This cannot be done with the regular arrangement of the planimeter, for when this is used with a projection microscope the tracing point is in the shadow of the tracing arm. An angulated tracing point projecting beyond the limit of the shadow cast by the tracing arm was attached and carefully calibrated for position. From the measurements thus gained, the actual area of the epidermis was calculated, and, sections having been cut at 8 microns, volume was determined. The values thus obtained were considered to be reliable estimates and not true volumes, for the shrinkage and distortion in fixation, embedding and cutting, the spherical aberration of the projection microscope and the errors inherent in the planimeter and the use of it render determination of true volume impossible. The advantage of this method is that it permits rapid examination of relatively large portions of tissue.

The volume of normal epidermis examined exceeded 0.004 cu. mm. in all but 3 instances; hence this was selected as the unit for comparison, and the number of mitoses observed in each specimen was converted to the number of mitoses per 0.004 cu. mm. The regenerating epidermis of wounds was similarly studied, and for it, too, the number of mitoses observed was expressed as the number per 0.004 cu. mm. Fisher's[8] small sample methods were employed in the statistical evaluation of the data.

RESULTS

Individual counts of mitoses and statistical comparisons are listed in tables 1 to 3. The mean values of mitotic activity in normal and regenerating epidermis for each four hour interval of the twenty-four hour period are presented graphically in the accompanying chart.

In normal epidermis mitotic activity displayed a diurnal rhythm, being minimum at 8 a. m. and maximum at 4 a. m. The difference between the means was statistically significant (table 2). Between 8 a. m. and 4 a. m. the mean rate of

4. Spain, K. C.: J. Exper. Med. **21**:193, 1915.

5. Spain, K. C., and Loeb, L.: J. Exper. Med. **23**:107, 1916.

6. Akaiwa, H.: J. M. Research **40**:371, 1919.

7. Arey, L. B.: Physiol. Rev. **16**:327, 1936.

8. Fisher, R. A.: Statistical Methods for Research Workers, ed. 7, Edinburgh, Oliver & Boyd, 1938.

TABLE 1.—*Individual and Average Numbers of Mitoses per 0.004 Cu. Mm. for Normal Epidermis*

Rabbit	8 a. m.	12 m.	4 p. m.	8 p. m.	12 p. m.	4 a. m.
A	1.6	4.1	4.9	9.2	6.9	11.4
B	5.0	1.6	14.8	8.7	24.3	5.3
C	6.4	11.0	11.3	7.0	12.0	15.6
D	10.4	3.2	6.2	17.1	10.9	12.0
E	9.2	9.6	6.7	4.5	4.0	14.8
F	6.8	3.8	2.8	5.3	6.5	8.9
H	2.2	16.9	4.5	6.9	6.8	4.6
J	1.8	1.1	7.0	6.2	3.8	8.5
K	5.8	14.1	6.0	5.1	4.6	6.5
L	7.7	4.3	3.7	1.3	7.0	4.0
	5.69	6.97	6.79	7.12	8.68	9.16
			For Regenerating Epidermis			
A	....	17.2	14.0	17.7	36.6	20.2
B	28.9	9.6	10.6	15.3	14.5	27.1
C	13.9	19.5	34.2	14.3	16.0	24.3
D	15.9	17.0	10.7	17.8	10.8	15.6
E	13.3	6.0	7.6	7.2	21.0	9.2
F	16.0	11.7	13.0	10.6	4.3	30.1
H	11.8	16.2	11.9	7.2	6.9	19.6
J	36.0	13.9	26.0	20.5	33.6	10.4
K	14.0	12.9	3.3	6.9	3.7	20.6
L	16.5	10.8	9.3	9.0	30.4	15.5
	18.47	13.48	14.06	12.65	17.78	19.26

TABLE 2.—*Statistical Data on the Significance of the Difference Between the Average Values of Mitotic Activity at Various Periods of Time*

Times Compared	Normal Epidermis			Regenerating Epidermis		
	Diff. ± S. E.	t	P	Diff. ± S. E.	t	P
8 a. m. vs. 12 m.	1.28 ± 1.909	0.640	0.6 - 0.5	4.99 ± 2.940	1.697	0.2 - 0.1
8 a. m. vs. 4 p. m.	1.10 ± 1.506	0.730	0.5 - 0.4	4.41 ± 4.019	1.097	0.3 - 0.2
8 a. m. vs. 8 p. m.	1.43 ± 1.636	0.873	0.4 - 0.3	5.82 ± 3.107	1,872	0.1 - 0.05
8 a. m. vs. 12 p. m.	2.99 ± 2.162	1.382	0.2 - 0.1	0.69 ± 4.828	0.142	0.9 - 0.8
8 a. m. vs. 4 a. m.	3.47 ± 1.635	2.121	0.05- 0.02	0.79 ± 3.449	0.228	0.9 - 0.8
12 m. vs. 4 p. m.	0.18 ± 2.094	0.085	>0.9	0.58 ± 3.176	0.182	0.9 - 0.8
12 m. vs. 8 p. m.	0.15 ± 2.189	0.068	>0.9	0.83 ± 2.067	0.401	0.7 - 0.6
12 m. vs. 12 p. m.	1.71 ± 2.606	0.656	0.6 - 0.5	4.30 ± 4.062	1.058	0.4 - 0.3
12 m. vs. 4 a. m.	2.19 ± 2.188	1.000	0.4 - 0.3	5.78 ± 2.506	2.305	0.05- 0.02
4 p. m. vs. 8 p. m.	0.33 ± 1.751	0.188	0.9 - 0.8	1.41 ± 3.316	0.425	0.7 - 0.6
4 p. m. vs. 12 p. m.	1.89 ± 2.250	0.839	0.5 - 0.4	3.72 ± 4.819	0.771	0.5 - 0.4
4 p. m. vs. 4 a. m.	2.37 ± 1.750	1.353	0.2 - 0.1	5.20 ± 3.606	1.441	0.2 - 0.1
8 p. m. vs. 12 p. m.	1.56 ± 2.339	0.666	0.6 - 0.5	5.13 ± 4.172	1.229	0.3 - 0.2
8 p. m. vs. 4 a. m.	2.04 ± 1.863	1.094	0.3 - 0.2	6.61 ± 2.681	2.464	0.06- 0.02
12 p. m. vs. 4 a. m.	0.48 ± 2.338	0.020	>0.9	1.48 ± 4.406	0.335	0.8 - 0.7

Diff. = difference between mean values; S. E. = standard error of the difference between mean values; t = significance ratio, obtained by dividing the difference between means by the standard error of that difference; P = an expression of the likelihood of occurrence by chance of a difference of the same magnitude as that observed. It is obtained from t according to the number of cases in the series compared. P of 0.01 means this chance is 1 of 100; P of 0.7, 70 of 100. Conventionally, P of 0.05 or less is interpreted as indicating statistical significance.

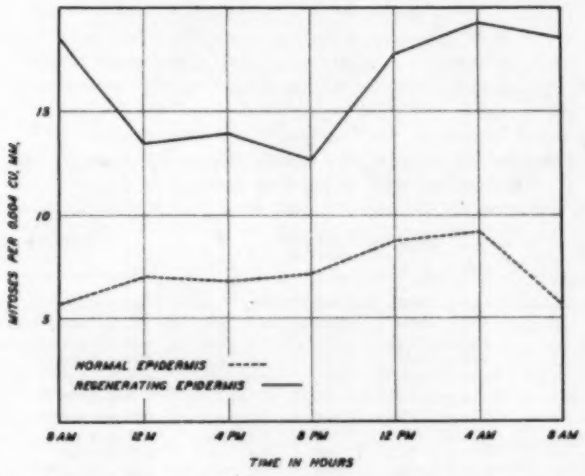
TABLE 3.—*Comparison of Mitotic Activity in Normal Epidermis with Mitotic Activity in Regenerating Epidermis (Mitoses per 0.004 Cu. Mm.)*

Time	Normal vs. Regenerating		Diff. ± S. E.	t	P
8 a. m.	5.69	18.47	12.78 ± 2.795	4.571	<0.01
12 m.	6.97	13.48	6.51 ± 2.236	2.911	<0.01
4 p. m.	6.79	14.06	7.27 ± 3.120	2.330	0.02
8 p. m.	7.12	12.65	5.53 ± 2.080	2.658	<0.01
12 p. m.	8.68	17.78	9.10 ± 4.307	2.112	0.05-0.02
4 a. m.	9.16	19.26	10.10 ± 2.516	4.013	<0.01
24-hour period	7.40	15.90	8.50 ± 1.203	7.061	<0.01

mitotic activity increased steadily, but none of the differences between these shorter intervals was statistically significant (table 2). It may be that with larger samples the differences would prove to be significant.

Regenerating epidermis displayed increased rate and diurnal periodicity of mitotic activity. The average rate of cell division for the twenty-four hour period was two and one-tenth times as great as in normal epidermis, ranging from one and five-tenth times as great at 8 p. m. to three and two-tenth times as great at 8 a. m. (table 1). At all periods the difference between means was statistically significant (table 3).

Mitotic activity in regenerating epidermis was minimum at 8 p. m. and maximum at 4 a. m. Both at 12 m. and 8 p. m. mitotic activity was significantly less than at 4 a. m. (table 2). Differences between other intervals were not significant. It may be noted that, unlike the means at other periods, the high mean of mitotic activity at 8 a. m. (table 1) was due to the high individual values for rabbits B and J, and that at 4 p. m., to the individual values for C and J. This may



Mean mitotic activity at four hour intervals for a period of twenty-four hours in equal volumes of normal and of regenerating epidermis.

account for the difference in form of diurnal periodicity in normal and in regenerating epidermis.

Thus mitotic activity showed a diurnal rhythm in both normal and regenerating epidermis, and its average rate was about twice as great in regenerating as in normal epidermis.

COMMENT

To the list of normal animal tissues showing diurnal periodicity of mitotic activity may be added the epidermis of the rabbit. As observations increase in number of species and variety of tissues examined, the probability increases that diurnal periodicity of mitotic activity is a fundamental character of growth by increase in number of cells. This is the first study reported in which specimens of normal tissue were obtained from the same animal at various times of the twenty-four hour period. Each of the 10 rabbits on which the experiment was completed showed such periodicity, but the time of maximum and minimum cell division was not always the same. In a majority of the animals, maximum division occurred

during the night, minimum during the day. The rabbit was like man, in whom mitotic activity is maximum at night [1d, f, g]; in rats and mice maximum cell division occurs during the day.[1b, e, h] These observations give additional support to the idea previously mentioned,[11] that external factors such as light and temperature are of little or no significance in determining mitotic activity and that internal factors such as functional and vegetative activities are of greater importance.

In regenerating epidermis mitotic activity was twice as great per 0.004 cu. mm. as in normal epidermis. Consideration of certain observations makes it probable that the rate of cell division was actually greater than this. Normal rabbit epidermis consisted of one or two layers of internally placed oval or multangular cells, in which mitoses were regularly seen, and a thin external layer of nucleated and non-nucleated squames. Regenerating rabbit epidermis seven days after a wound was made commonly showed five to ten layers of cells exhibiting the usual stratification of thick epidermis. Mitotic figures were common in the basal cells and rare elsewhere. Thus, of 0.004 cu. mm. of normal epidermis, a main portion consisted of cells in which mitosis occurred; but, of the same volume of regenerating epidermis, a major portion consisted of cells in which mitotic figures were rarely or never seen. The true rate of division in those cells of regenerating epidermis commonly undergoing mitosis must have been much greater than the rate estimated per 0.004 cu. mm. However, there being no way of accurately estimating the true rate, it can only be indicated that mitotic activity probably was much more than twice as great in regenerating as in normal epidermis. That controlled growth in developing or regenerating tissues may equal or exceed uncontrolled neoplastic growth is known (Schrek [9]; Eveleth and Wetzel [10]; Aub and Wislocki [11]). The importance of considering the mode of growth is thereby emphasized.

Diurnal periodicity of mitotic activity was observed in regenerating rabbit epidermis. The time of maximum activity was the same as in normal epidermis, 4 a. m. That of minimum activity was not, being 8 p. m. in the regenerating and 8 a. m. in the normal epidermis. The high mean value for mitotic activity in regenerating epidermis at 8 a. m. was due to individual values for rabbits B and J (table 1), which were about twice as great as any of the others. The distribution of individual values in the significantly different maximum and minimum periods was not abnormal (table 1). This emphasizes the importance of large samples. From these data it may be stated that diurnal periodicity was exhibited in regenerating epidermis but it cannot be concluded that the curve representing periodicity was the same as or different from that of normal epidermis.

To compare mode as well as rate of normal, hyperplastic and neoplastic growth, material from the same species of animals should be examined. To the present this is not possible, for the only available studies of mitotic activity at various intervals of a twenty-four hour period are on normal epidermis and epidermal carcinoma of male CBA mice [3] and on normal and hyperplastic regenerating epidermis of male albino rabbits. These studies show that in normal epidermis of both mice and rabbits there was diurnal mitotic rhythm. In epidermoid carcinoma of the mouse the average mitotic activity was one and eight-tenths times normal; in the regenerating epidermis of the rabbit it was two and one-tenth (probably much greater) times normal. Diurnal periodicity was absent from the carcinoma and present in the regenerating epidermis. Studies of the diurnal rate

9. Schrek, R.: Am. J. Path. **12**:525, 1936.
10. Eveleth, M. S., and Wetzel, N. C.: Cancer Research **1**:721, 1941.
11. Aub, J. C., and Wislocki, G.: Cancer Research **3**:120, 1943.

and rhythm of mitosis in hyperplastic and neoplastic tissues of older mice, rats and rabbits are in progress. The results will help to determine whether loss of diurnal periodicity of mitotic activity is a characteristic of neoplastic growth alone.

SUMMARY

Wounds were made in the skin of each of 10 young male albino rabbits at 8 a. m., 12 m., 4 p. m., 8 p. m., 12 p. m. and 4 a. m. Each wound was removed exactly seven days after it was made. The piece of skin removed to make the wound served as the control, demonstrating mitotic activity in normal epidermis. The regenerating epidermis of the wounds was studied as an example of non-neoplastic hyperplasia.

Mitotic activity in normal epidermis displayed diurnal periodicity, being minimum at 8 a. m., 5.96 mitoses per 0.004 cu. mm. of epidermis, and maximum at 4 a. m., 9.16 mitoses per 0.004 cu. mm. The difference is statistically significant.

In regenerating epidermis the average rate of mitotic activity was increased to twice that of normal epidermis. There was periodicity of mitotic activity, with a minimum rate of 12.65 per 0.004 cu. mm. at 8 p. m. and a maximum rate of 19.26 per 0.004 cu. mm. at 4 a. m. The difference is statistically significant.

This and a preceding study show that in both non-neoplastic hyperplasia, exemplified by regenerating epidermis of cutaneous wounds in rabbits, and neoplastic hyperplasia, exemplified by epidermoid carcinoma of mice, the average rate of mitotic activity is about twice that of the respective normal epidermis but that diurnal periodicity of mitotic activity is present in the regenerating epidermis and absent in carcinoma.

Case Reports

ACUTE INTERSTITIAL NEPHRITIS WITH UREMIA

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Acute interstitial nephritis is encountered occasionally as an incidental finding at autopsy in cases of sepsis due to infection with the hemolytic streptococcus, such as scarlet fever, and less commonly in cases of sepsis due to other acute infections. No clinical evidence of disturbed renal function can ordinarily be correlated with the lesion, and the glomeruli are generally found to be normal. The case to be reported now presented at autopsy the classic picture of interstitial nephritis; death resulted from uremia. A consideration of the mechanism by which uremia might have developed is therefore of interest.

A 33 year old white man was admitted to the Station Hospital, Fort Lewis, Wash., on Sept. 24, 1942, complaining of a sore throat, chills and general aching. The essential physical findings at this time consisted of greatly swollen, red tonsils and swollen adjacent gingiva. Smears from the tonsils revealed no significant organisms; material for cultures was not taken. Local medication was of no avail; severe weakness, restlessness and stupor developed within two or three days, with a temperature of 105 F. A blood culture at this time yielded an abundant growth of the beta hemolytic streptococcus, and three subsequent blood cultures were also positive for the hemolytic streptococcus. Slight jaundice was noted, which progressively increased in intensity up to an icterus index of 73. The urine on entrance contained albumin (2+) and a few casts; the specific gravity was 1.021; it dropped to between 1.011 and 1.013 and remained fixed at this level until his death. Oliguria developed terminally. Large amounts of albumin and many red blood cells and casts were present in almost every specimen. The hemoglobin was 15 Gm. per hundred cubic centimeters but gradually dropped to 10 Gm. before death. The erythrocyte count was 4,900,000 per cubic millimeter, dropping gradually to 2,900,000. There was polymorphonuclear leukocytosis, the count varying from 18,000 to 34,000, with a shift to the left in the Schilling test. The spinal fluid was not significantly altered. Sulfadiazine was administered, the blood level varying from 10.6 to 16.7 mg. per hundred cubic centimeters. Because of the urinary findings and the stupor the blood nonprotein nitrogen was determined and was found to be 111 mg. per hundred cubic centimeters. This increased to 226 mg. the day before his death. Dyspnea, rales in the chest, tachycardia and cardiac murmurs developed; the toxemia and stupor progressively increased in severity, and in spite of the administration of fluids, sulfadiazine, oxygen and other agents, the patient died on October 7. The clinical diagnosis was acute tonsillitis, hemolytic streptococcus septicemia, acute glomerular nephritis with uremia, and severe icterus.

At autopsy the kidneys were much swollen, the right weighing 345 Gm. and the left 310 Gm. They were soft; the tense capsules stripped easily, leaving a smooth purple-brown surface studded by occasional pinpoint size hemorrhages. When cut, the cortex was found swollen to 9 mm. in thickness; the cut surfaces were purple-brown, presented small patchy areas of dark red injection and were studded by occasional pinpoint size hemorrhages. The pelvis and calices were normal in size and appearance, and their mucosa was light gray-red and smooth. The ureters and the urinary bladder were grossly normal. The spleen was markedly swollen, weighing 560 Gm.; it was soft, the edges were rounded and the capsule was slightly granular and dark purple-gray; the cut surfaces were deep purple-red, with indistinct follicles and trabeculae, and the soft pulp could be easily scraped off from the cut surface. The liver was also swollen; its weight was 2,700 Gm.; the edges were rounded; the capsule was light yellow-brown and smooth; the consistency was moderately firm; the cut surfaces were homogeneous light yellow-brown to gray-brown and the lobulation was indistinct except for the centers of the lobules, which formed tiny yellow-brown patches. The gallbladder and the extrahepatic bile ducts were normal throughout. The heart weighed 380 Gm., the myocardium was very soft and friable and the chambers were dilated; otherwise the heart was normal. The lungs were markedly edematous and congested and were studded by focal areas of consolidation. The brain weighed 1,640 Gm. and was edematous and congested. The mucosa of the entire gastrointestinal tract was studded by occasional pinpoint size hemorrhages, and there was a

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dark red to black bloody intestinal content. The adrenal glands together weighed 15 Gm.; the cortex was swollen to 3 mm. and was light gray; the medulla was hyperemic. The skin, the scleras, the aortic intima and the dura mater were icteric. The other organs were essentially normal.

Microscopic sections through several areas of both kidneys when stained with hematoxylin-eosin and by Masson's trichrome and Gram-Weigert methods revealed extensive interstitial infiltrations of plasma cells, located chiefly between the tubules but also around some of the glomeruli (fig. 1). Mixed with the plasma cells were occasional lymphocytes and histiocytes and few polymorphonuclear and eosinophil leukocytes. The infiltrations were focal and

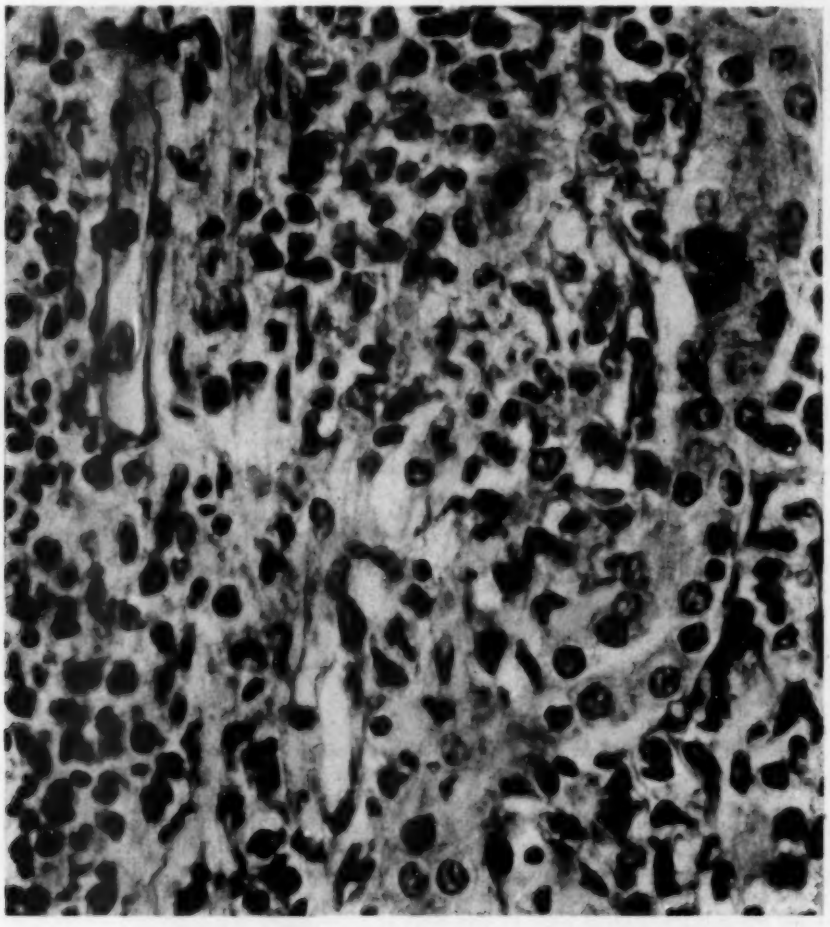

Fig. 1.—Acute interstitial plasma cell nephritis; hematoxylin and eosin; × 500.

were located chiefly in the vicinity of Henle's loops. They were often so abundant as to obscure the tubules, resulting in atrophy of some of them, and were usually associated with areas of marked hyperemia. The infiltrations surrounding some of the glomeruli were less prominent.

Many glomeruli were swollen; the glomerular tufts often showed slight swelling of all layers; some were infiltrated by a few lymphocytes and polymorphonuclear leukocytes. Bowman's space was often widened and contained granular debris. The parietal layer of Bowman's capsule, although often flat, was frequently much swollen so that the cells appeared cuboidal (fig. 2); occasionally the cytoplasm of the swollen cells was granular and vacuolated, and the nuclei were pyknotic. A rare glomerulus was necrotic, being composed of structureless material showing beginning organization by young fibroblasts.

The epithelium of most of the convoluted tubules was swollen, granular, vacuolated and pale staining, and some of the nuclei showed karyolysis. A few of the convoluted tubules were by contrast fairly intact. The lumens of many of the tubules were dilated; in many Henle's loops and distal convoluted tubules there were casts of precipitated hemoglobin; a great many of the collecting tubules contained epithelial and blood casts, which were occasionally bile stained. Occasional tubules contained polymorphonuclear leukocytes in their lumens; rare sulfadiazine crystals were also seen. In the medulla the collecting tubules were everywhere widely separated by edema. In the cortex there was an occasional small hemorrhage. The

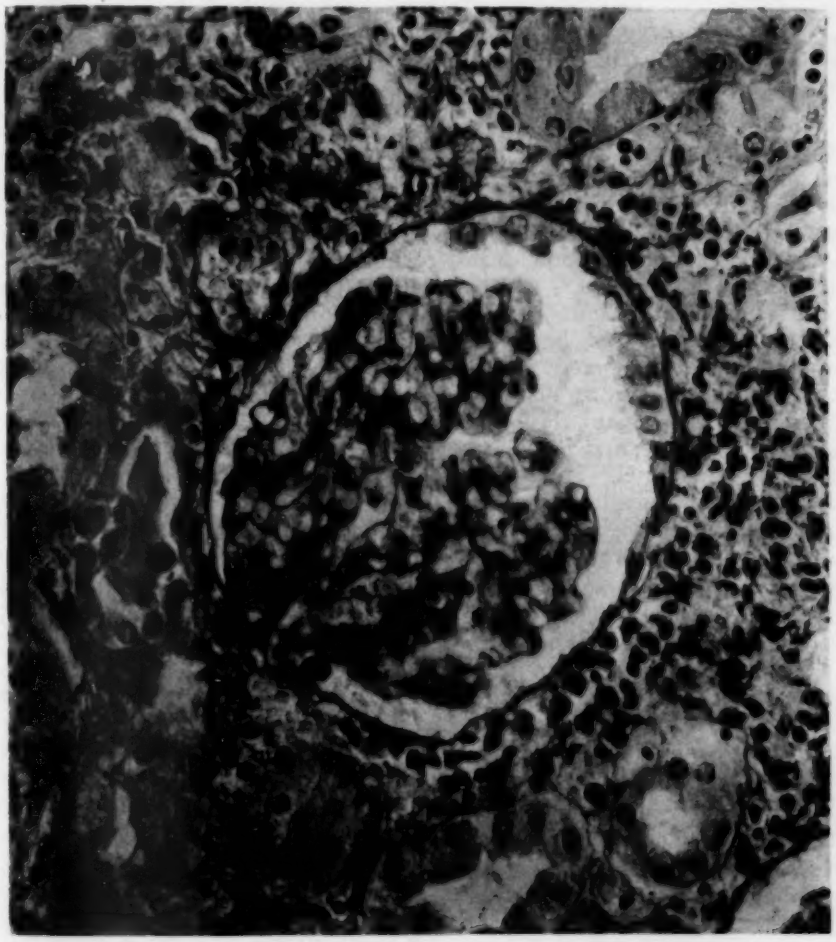

Fig. 2.—Marked swelling of the parietal layer of Bowman's capsule; hematoxylin and eosin; × 250.

arterioles showed no changes. The renal pelvis was normal. Gram-Weigert staining revealed no bacteria.

The spleen was also of interest; many areas were seen to be infiltrated by large numbers of plasma cells. The sinusoids were widened and filled with blood and polymorphonuclear leukocytes; their lining endothelium was somewhat swollen. The follicles were small and were infiltrated by plasma cells. Occasional small foci of necrosis were seen. In the liver many of the portal areas contained abundant collections of polymorphonuclear leukocytes mixed with some lymphocytes and plasma cells. The sinusoids contained similar cells as well as much granular material. The Kupffer cells were much swollen and often filled the sinusoids. The liver cell cords were swollen and granular, with eosinophilic cytoplasm and

many pale-staining or pyknotic nuclei. Many intracellular and intercellular bile casts were seen. The central veins were dilated. The myocardium showed severe degenerative changes, hyperemia and perivascular edema which had produced loosening of the adventitia of some of the arterioles. The lung was hyperemic and edematous, with occasional foci of fibrin, red cells, alveolar phagocytes and eosinophilic hyaline bands in the alveolar spaces. The adrenal glands were markedly hyperemic and edematous. In the mucosa of the stomach and intestine there were occasional small hemorrhages. The brain also was hyperemic and edematous; the nuclei of some of the ganglion cells were pyknotic.

The anatomic and clinical diagnosis was: acute interstitial nephritis; uremia; septic hyperplasia of the spleen with plasma cell infiltrations and focal areas of necrosis; toxic hepatitis; icterus; parenchymatous degeneration of the myocardium; passive congestion of the lungs with bronchopneumonia; hyperemia and edema of the brain and of the adrenal glands; petechial hemorrhages in the mucosa of the gastrointestinal tract with bloody intestinal content; hemolytic streptococcus septicemia.

COMMENT

Interstitial nephritis in cases of sepsis due to infection with the hemolytic streptococcus and to other acute infections is characterized by interstitial infiltrations chiefly of plasma cells in the kidneys without significant changes in the glomeruli and without disturbances of renal function during life. In some cases the infiltrations are composed of polymorphonuclears, eosinophils or lymphocytes; in others only severe inflammatory edema is seen. Whatever the cell type in the kidneys may be, identical cells are often found infiltrating other organs also. In addition to acute infections, toxic processes, burns, poisonings, allergic states, hepatic damage (the hepatorenal syndrome) and transfusion or other hemolytic reactions may be associated with the lesion. The incidence of interstitial nephritis appears to be increasing in recent years; the sulfonamide compounds are thought to be a factor. Councilman [1] was the first to describe clearly the plasma cell variety of interstitial nephritis. Kimmelstiel,[2] Huebschmann,[3] Mallory and Keefer [4] and others have regarded interstitial nephritis as a hyperergic reaction of the sensitized kidney to the particular toxin involved. There is some experimental evidence to support this view (Spink [5]; Horn [6]; Ahlström.[7]).

Although occasional authors have mentioned that patients with this lesion may die of uremia, relatively few cases of acute interstitial nephritis (without associated glomerulonephritis) with uremia have been reported. Kimmelstiel,[2] Jochweds and Bajer [8] and Helwig and Orr [9] have reported several cases of interstitial nephritis following miscellaneous types of infections, burns, hepatic damage and hemolytic reactions. Noble [10] reported a case of the plasma cell variety with uremia following scarlet fever; Noble's case is the only one that can be found that closely resembles the case reported here. In all of these cases the glomeruli were found to be practically normal. Several theories attempting to explain the mechanism of the uremia may be considered:

Mechanical Effect.—Bell,[11] Noble [10] and others have expressed the belief that the uremia in these cases may result from severe involvement of the kidney by the infiltrations, with compression of the blood vessels or tubules or both by the massive exudate or by the abundant edema and blockage of tubular lumens by

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2. Kimmelstiel, P.: Am. J. Path. **14**:737, 1938.
3. Huebschmann, P.: Klin. Wchnschr. **8**:2221, 1929.
4. Mallory, G. K., and Keefer, C. S.: Arch. Path. **32**:334, 1941.
5. Spink, W. W.: Arch. Int. Med. **59**:65, 1937.
6. Horn, H.: Arch. Path. **23**:71, 1937.
7. Ahlström, C. G.: Acta path. et microbiol. Scandinav., 1936, supp. 29, p. 1.
8. Jochweds, B., and Bajer, I.: J. d'urol. **48**:42, 1939.
9. Helwig, F. C., and Orr, T. G.: Arch. Surg. **24**:136, 1932.
10. Noble, J. F.: Arch. Path. **2**:612, 1926.
11. Bell, E. T.: Am. J. Path. **13**:497, 1937; **14**:691, 1938.

casts or precipitated materials. In the present case such intrarenal hydronephrosis was perfectly possible; infiltrations, edema and an abundance of casts were all present.[11a]

Toxic Effect of Sulfadiazine.—The toxicity of sulfonamide compounds is becoming a problem of considerable importance. Comparatively few postmortem studies have been made in this field (Merkel and Crawford [12]; Lederer and Rosenblatt [13]). Some of the findings which have been considered significant are focal areas of necrosis in many organs; round cell infiltration of blood vessels, especially veins, and interstitial nephritis. In the present case the interstitial nephritis and the foci of necrosis in the spleen may indicate toxicity of the sulfonamide compound, especially in view of the finding of occasional sulfadiazine crystals in the kidneys. Whether the uremia resulted from such toxicity is difficult to decide. Such a toxic action should be sharply differentiated from the commonly occurring mechanical blockage of tubules by an abundance of precipitated crystals of a sulfonamide compound; the latter did not occur in the present case.

Hyperergic Inflammation.—As stated, a number of authors regard interstitial nephritis as a hyperergic reaction in a kidney that has become sensitized to a given toxin. Kimmelstiel [2] expressed the belief that in cases with uremia the latter is also on an allergic basis. The concept of the allergic nature of glomerulonephritis has received considerable attention in recent years. It is difficult from a purely morphologic standpoint to see an allergic mechanism in such cases as the one reported here; however, the glomeruli in this case are actually not all perfectly normal; the marked swelling of the parietal layer of Bowman's capsule in some of them is of interest. Others have reported occasional similar findings. (Huebschmann [3]; Russell [14]). The early minimal findings in acute glomerulonephritis described by McGregor [15] and Bell [11] are also of interest. The marked swelling of the parietal layer of Bowman's capsule in the present case may represent an early phase of hyperergic inflammation as described by Klinge,[16] also by Pagel [17] and Rössle.[18] The intense edema may also be part of this picture. There is none of the characteristic fibrinoid necrosis of the collagen, however. The morphologist must be cautious in interpreting the histologic aspect of allergic inflammation.

Prerenal Azotemia.—The physiologic and clinical concept of extrarenal or prerenal azotemia without anatomic changes in the kidney is receiving considerable attention (Fishberg [19]). Shock is a good example; when the filtration pressure in the renal blood vessels is lowered, a uremic state may supervene. Van Slyke and associates [20] have demonstrated how sensitive are the glomeruli to renal blood flow. Such a mechanism may occur in many types of severe metabolic disturbances, namely: shock, hemorrhage, alkalosis, acidosis, severe dehydration, diabetic coma, the hepatorenal syndrome. Further, almost any form of injury may cause

11a. Lieutenant Colonel Balduin Lucké called attention to the numerous casts of precipitated hemoglobin in the renal tubules in this case, presumably from the hemolysis caused by an overwhelming infection with the hemolytic streptococcus. Other hemolytic states, such as transfusion reactions, blackwater fever and the "crush syndrome" (wherein myohemoglobin is released from crushed muscles), are associated with similar anatomic and clinical pictures.

12. Merkel, W. C., and Crawford, R. C.: J. A. M. A. **119**:770, 1942.

13. Lederer, M., and Rosenblatt, P.: J. A. M. A. **119**:8, 1942.

14. Russell, D. S.: A Classification of Bright's Disease, Medical Research Council, Special Report Series no. 142, London, His Majesty's Stationery Office, 1929, p. 80.

15. McGregor, L.: Am. J. Path. **5**:559, 1929.

16. Klinge, F.: Virchows Arch. f. path. Anat. **286**:344, 1932; Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1933.

17. Pagel, W.: Acta med. Scandinav. **84**:422, 1935.

18. Rössle, R.: Wien. klin. Wchnschr. **45**:609 and 648, 1932; Klin. Wchnschr. **12**:574, 1933.

19. Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, Lea & Febiger, 1939.

20. Van Slyke, D. D.; Rhoads, C. P.; Hiller, A., and Alving, A. S.: Am. J. Physiol. **109**:336, 1934; **110**:387, 1934.

the glomerular filter to become permeable to albumin and even red cells without leaving any anatomic trace. Kimmelstiel [2] expressed the belief that cases of interstitial nephritis with uremia represent cases of prerenal azotemia on an allergic basis. Here again, however, the morphologist must be cautious in dealing with what is almost entirely a problem in pathologic physiology.

SUMMARY

Acute interstitial nephritis is usually characterized by interstitial plasma cell infiltrations in the kidneys without notable changes in the glomeruli and usually without disturbances of renal function during life. The plasma cell variety is usually seen in hemolytic streptococcus sepsis; other types of interstitial infiltration (polymorphonuclear, eosinophilic, lymphocytic, inflammatory edema) are seen in other toxic states. The lesion is considered to represent a hyperergic reaction of the sensitized kidney to a given toxin, although a possible relation to the sulfonamide compound used in therapy is receiving attention. Uremia resulting from this lesion is relatively uncommon. In the case reported in the foregoing pages death resulted from uremia. The various theories explaining the genesis of the uremia (mechanical effect; toxicity of the sulfonamide compound; hyperergic inflammation; prerenal azotemia) are discussed. In the present case the theory that the lesion is due to a mechanical effect appears most logical on morphologic grounds alone, although there is some basis for considering each of the other three.

FATAL METHYL BROMIDE POISONING

J. W. Miller, M.D., Bethesda, Md.

In 1932 Le Goupil [1] reported the insecticidal properties of methyl bromide. Prior to that date the use of methyl bromide was relatively restricted. It was used chiefly in the chemical industry in the preparation of methyl compounds and dyes and in the pharmaceutical industry in the preparation of antipyrine, and it was used in the manufacture of fire extinguishers. In the last it was usually mixed with carbon tetrachloride. European publications contain a few reports of fatal poisoning resulting from exposure in these industries.

Since 1935 the use of methyl bromide as a fumigant, principally for food stuffs, has steadily increased because of its effectiveness at moderate concentrations, because it kills most insects at almost all stages of their development and because of the ease with which it can be handled. While few accidents have occurred incident to its use as a fumigant, its hazardous nature should not be minimized.

The case of fatal poisoning to be reported presents a definite history of exposure to methyl bromide alone.

An itinerant, in search of a place to sleep, forcibly entered a refrigerator car filled with baled alfalfa hay in process of being fumigated with methyl bromide and made his bed on the ice racks in the empty ice bunker at the end of the car. He entered through the car roof, leaving the hatch open behind him. The fumigant was introduced at about 3:30 p. m. It is believed that the man entered the car about fourteen hours later, as he said he entered before dawn the next day. The ice racks in which he made his bed were about 12 inches (30 cm.) above the bunker floor, and as no blowers were used, the concentration was undoubtedly heavy, since the end compartments are difficult to fumigate. Five pounds of methyl bromide were used to fumigate the car, approximately 2 pounds per thousand cubic feet (8,160 parts per million in air or 32 mg. per liter). Noise caused by his convulsions revealed his presence about five and a half to seven and a half hours after he entered the car.

The man was unconscious when removed from the car. Respiration and heart action were hardly noticeable. Oxygen was administered, and other medication described as "acid neutralizers and stimulants" was applied. Following this treatment, he regained consciousness but not muscular control. The action of his eyelids in winking was described by a witness as "similar to that of a 2 year old child." He complained of dryness of the throat and continuously asked for water. The musty odor associated with methyl bromide was detected on his breath and body. This odor was most marked when he was removed from the car but was present throughout the entire time he survived. Attempts were made to type his blood for transfusion but the blood failed to react; however, on the following day the same sample of blood was easily typed. He died eighty hours after his removal from the car.

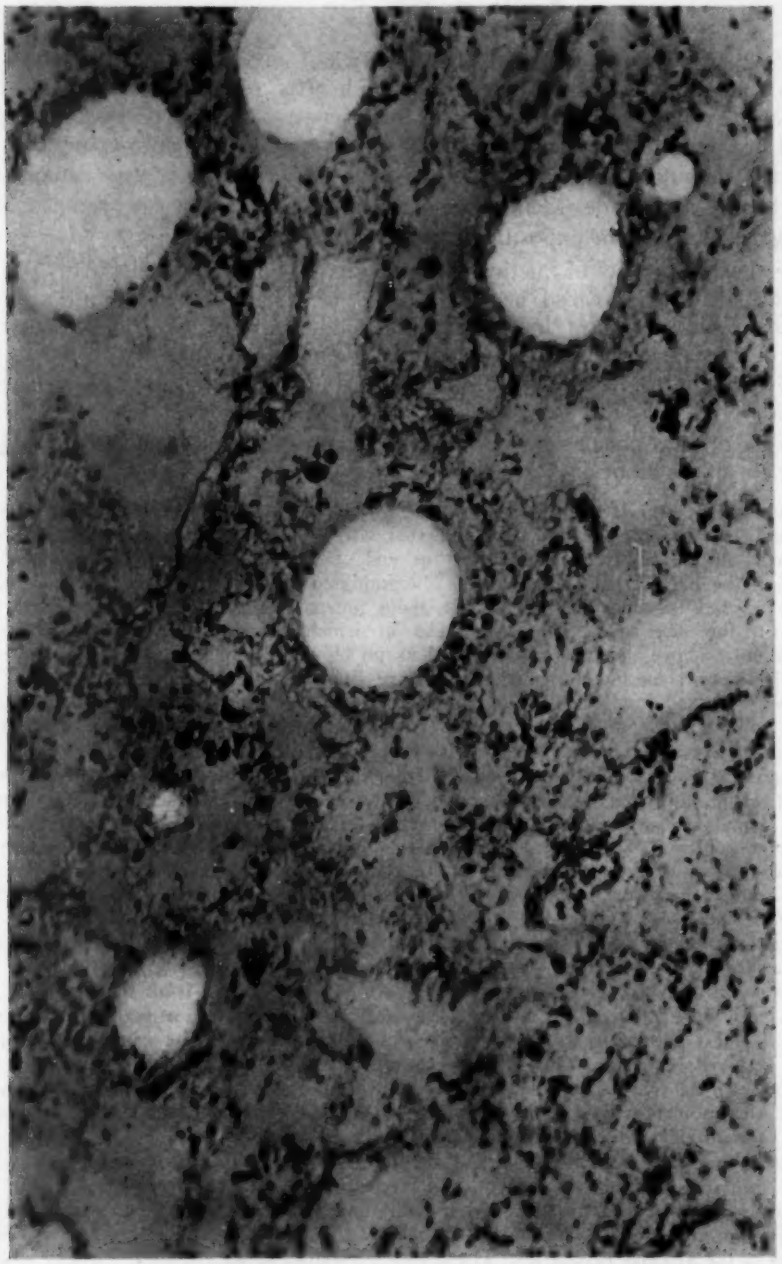
The organs were removed at autopsy, preserved in solution of formaldehyde and shipped intact by the local authorities to this laboratary for examination. The material, for the most part, was received in a fair state of preservation but all of the organs were stained a deep bluish green which was more intense in certain tissues such as the renal cortex and the mucosa of the esophagus and stomach. It is thought the patient had received intravenous injections of methylene blue in the course of treatment.

The right lung weighed 816 Gm. and the left 630 Gm. The gross appearance of both lungs was similar. Crepitus was absent, and on section the cut surface exuded a thin muddy fluid. Microscopically, many sections from both lungs showed extensive exudation of serum into the alveolar spaces and a swelling of the cells of the alveolar walls so great as to obliterate the outlines of the alveolar capillaries. These very edematous areas gradually merged with zones in which the serum in the alveolar spaces and the swelling of the walls were lessened and the alveolar capillaries were filled with red blood cells. In some of the sections acute focal

From the Division of Industrial Hygiene, National Institute of Health, United States Public Health Service.

1. Le Goupil, M.: Rev. path. veg. et ent. agric. de France **19**:169, 1932.

pneumonitis was present. Here, alveoli were filled with large numbers of polymorphonuclear leukocytes and serum, and alveolar capillaries were congested. An occasional alveolus or small groups of alveoli were filled with denser purulent exudate. Some bronchi were filled with



Pulmonary edema following exposure to methyl bromide.

pus, others were essentially normal, while still others, chiefly in the edematous areas, contained mononuclear cells with scanty cytoplasm. These cells may have been desquamated alveolar epithelium since attached epithelium was relatively scanty in these areas.

A considerable variation was observed in the staining qualities of the erythrocytes, grading from well defined, normal-appearing cells to more "shadow" (achromic) cells. This phenomenon occurred without regard to the adjacent histologic structure. A large amount of amorphous brown pigment, largely intracellular, was noted in all of the sections. It was present in the cells of the alveolar walls, in macrophages in the alveolar spaces, in the intima of the blood vessels and in phagocytes in the lumens of blood vessels as well as extracellularly. A part of this pigment was birefringent to polarized light but none reacted with ferrocyanide. This is the usual so-called "formaldehyde pigment" seen in partially hemolyzed postmortem material.

The liver weighed 1,900 Gm. The margins were somewhat rounded, the capsule was tense and the organ was firm in consistency. The cut surface presented no abnormal features. Microscopically, the sinusoids were moderately distended with red blood cells. The cells of the cords were of normal size or slightly swollen. The cytoplasm was granular and fairly dense, and throughout the entire lobule it contained few to many fine fat droplets. The nuclei varied considerably in size, and a few appeared as thin chromatin rings with clear centers.

The spleen weighed 230 Gm. The splenic follicles were small, and the pulp and sinuses were greatly distended with blood.

The kidneys, the heart, the adrenal glands, the testes, the skin, the skeletal muscle and the esophagus showed no significant changes. Postmortem desquamation of the surface cells of the mucosa was noted in the stomach and in the small and the large intestine. The deeper layers were of usual appearance.

The brain weighed 1,466 Gm. Postmortem softening due to poor fixation rendered microscopic examination useless.

SUMMARY AND COMMENT

A man was accidentally exposed to methyl bromide in a refrigerator car filled with hay. The concentration of methyl bromide was approximately 8,160 parts per million in air (2 pounds per thousand cubic feet) fourteen hours before his entrance into the car. The duration of exposure was between five and a half and seven and a half hours. He was unconscious when found but was revived by administration of oxygen and other medication not specified. Muscular control was impaired throughout the entire time he survived, which was approximately eighty hours after the end of exposure.

The most significant pathologic changes were extensive pulmonary edema, seropurulent pneumonia (small foci), acute bronchitis and early fatty degeneration of the liver. Identical pulmonary changes in man have been reported by Goldschmidt and Kuhn,[2] Meixner[3] and Duvoir and co-workers.[4] In these cases, however, exposure was due to the use of methyl bromide as a fire extinguisher fluid. Similar changes in the lungs were also observed in experimental animals by Irish,[5] Joachimoglu[6] and Beyne and Goett.[7] There was moderate fatty degeneration of the liver of the fine droplet variety. Since the victim lived more than three days, and as the homologue methyl chloride has been reported to produce fatty degeneration of the liver,[8] it seems probable that this change is due to methyl bromide poisoning. However, the major cause of death in this case was pulmonary edema.

2. Goldschmidt, E., and Kuhn, E.: Zentralbl. f. Gewerbehyg. **8**:28, 1920.
3. Meixner, K.: Beitr. z. gerichtl. Med. **8**:10, 1928.
4. Duvoir, M.; Fabre, R., and Layani, F.: Bull. et mém. Soc. méd. d. hôp. de Paris **53**:1540, 1937.
5. Irish, D. D.; Adams, E. M.; Spencer, H. C., and Rowe, V. K.: J. Indust. Hyg. & Toxicol. **22**:218, 1940.
6. Joachimoglu, G.: Deutsche med. Wchnschr. **56**:785, 1930.
7. Beyne, J., and Goett, M.: Arch. de méd. et pharm. nav. **124**:409, 1934.
8. Kegal, A. H.; McNally, W. D., and Pope, A. S.: J. A. M. A. **93**:353, 1929.

ONCOCYTOMA OF THE PAROTID GLAND

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Stout [1] recently reviewed the literature on the origin, location and histologic character of oncocytes. Oncocytes are epithelial cells resembling those of the organ in which they are found and tend to be large with acidophilic granular cytoplasm. Their nuclei resemble the nuclei of the cells of the parent organ. They give rise to nodular hyperplasia and at times are the source of a tumor known as oncocytoma. Their usual sites are the anterior and posterior lobes and the stalk of the hypophysis, the salivary glands, the thyroid and parathyroid glands, the pancreas, the liver, the uterine tube, the testes and the bronchi.[2] The case to be reported is a classic one of oncocytoma of the parotid gland:

REPORT OF A CASE

A white man aged 76 was first seen Nov. 17, 1941 for a carcinoma of the left cheek. There was a small painless lump beneath the left ear which had been present for several months and had been gradually increasing in size. This nodule measured 2 cm. in diameter and was located apparently in the tail of the parotid gland. It was not attached to skin or bone, was freely movable and felt quite firm. The patient refused surgical removal until Feb. 13, 1943. The preoperative diagnosis was mixed tumor of the parotid gland.

The tumor was widely excised, with a generous portion of the normal salivary gland. Within this gland there was a firm, smooth, sharply circumscribed, apparently encapsulated tumor which on section was homogeneous and grayish red and measured 2 cm. in diameter. The entire tumor was fixed in Zenker's fluid and was stained with the phosphotungstic acid–hematoxylin, Wilder, hematoxylin-eosin, eosin–methylene blue, Verhoeff and Masson trichrome stains. It was sectioned serially.

The tumor had an incomplete connective tissue capsule. Around its periphery were collections of adult lymphocytes, which were intimately associated with the tumor. The tumor cells were all similar in appearance, were arranged in columns and at times formed pseudoacini. They were large and had bright acidophilic granular cytoplasm. Their nuclei, for the most part, were round or vesicular and had moderately prominent nucleoli. These cells bore a superficial resemblance to liver and adrenal gland.[3] Mitotic figures were extremely rare. Running between the columns was a delicate network of small blood vessels, and only a small amount of connective tissue supported the tumor. The special stains gave little additional information except that the eosin–methylene blue stain was excellent for nuclear detail. The Masson trichrome stain showed the cytoplasm to be composed of rather coarse greenish granules, which in some cells were interspersed with golden yellow granules of about the same size. Coalescence of these granules did not occur. Ducts within the tumor were rare except in one area, where there was a small collection of them. Some of the cells of these ducts resembled oncocytes, and one of these blended with the tumor. The parotid gland surrounding the tumor was normal.

COMMENT

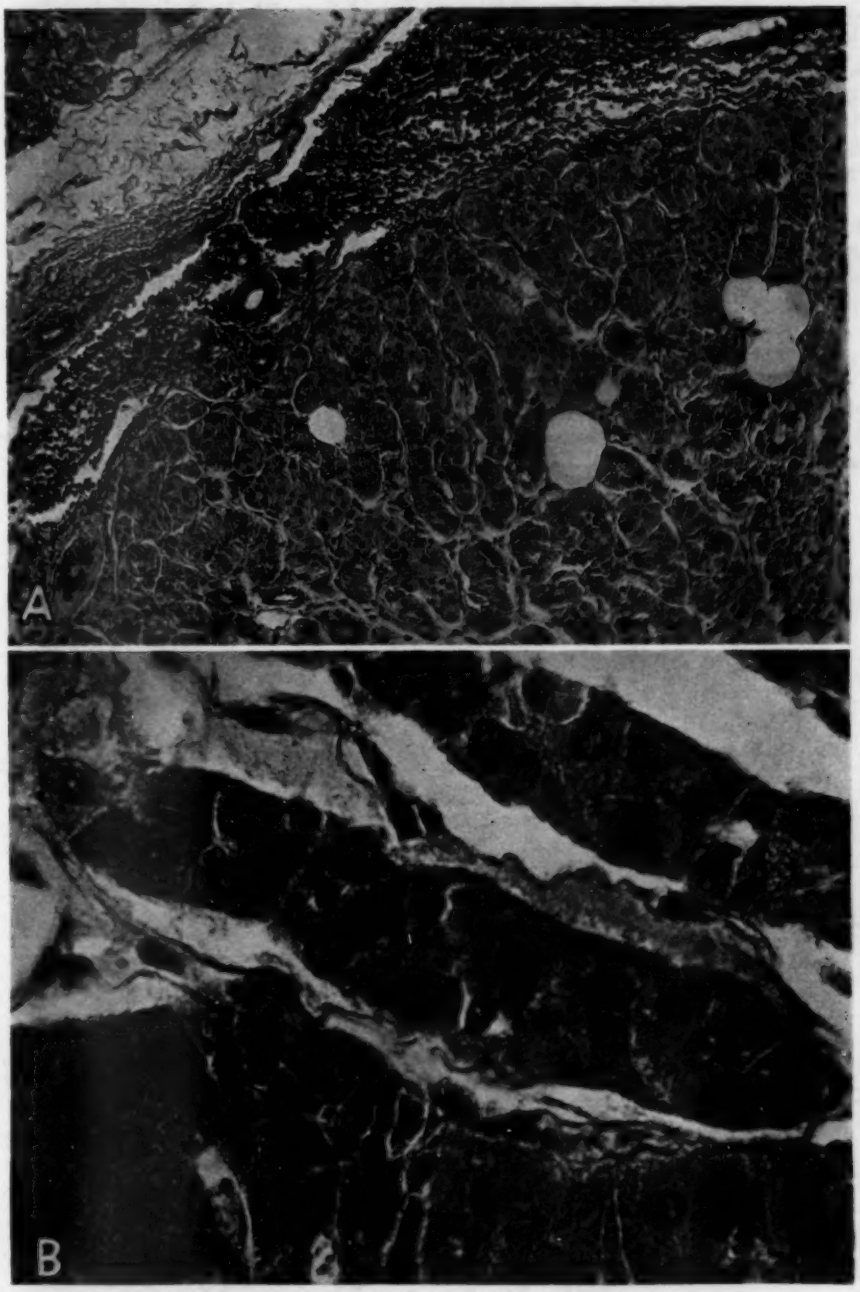
The accompanying table represents the few well documented cases of oncocytoma of the salivary gland, but it is possible that there are others masquerading under other names or incorrectly classified. The table shows that patients with oncocytoma are in the older age group (average age, 72 years). Hamperl [2a] demonstrated that the presence of oncocytes and nodular hyperplasia in the salivary glands is common in aged persons. Oncocytes have not been found in patients under 20 but are found with increasing frequency in those between 20 and 60, and after

From the Department of Pathology, Ellis Fischel State Cancer Hospital.

1. Stout, A. P.: Arch. Path. **35**:803, 1943.

2. Hamperl, H.: (*a*) Ztschr. f. mikr.-anat. Forsch. **27**:26, 1931; (*b*) Virchows Arch. f. path. Anat. **282**:724, 1931; (*c*) **291**:704, 1933; (*d*) **298**:327, 1936.

3. McFarland, J.: Am. J. M. Sc. **174**:362, 1927.



A, oncocytoma; × 115; eosin-methylene blue stain. Note arrangement of the tumor cells, lymphocytic infiltration and incomplete connective tissue capsule.

B, oncocytoma; × 900; eosin–methylene blue stain. This demonstrates the granularity of the cytoplasm and the characteristic nuclei.

Recorded Cases of Oncocytoma of the Salivary Glands

Author	Patient		Tumor's Duration	Site	Gross Aspects				Microscopic Aspect	Follow-Up
	Sex	Age			Size	Consistency	Color	Attachment to Skin		
McFarland [8]	M	71	3 yr.	Right parotid	4.5 × 4 × 2 cm.	Firm	Dark brownish red	None	Characteristic	New tumor 12 yr. after surgical removal; death 6 yr. later, not due to tumor
Gruenfeld and Jorstad [9]	F	68	3 yr.	Right parotid	3 × 4 cm.	Firm	Brownish red	None	Characteristic	Followed for 7 yr.; no recurrence; death unrelated to tumor
Steinhardt [7a]	M	67	No data	Right parotid	No data	No data	No data	No data	Characteristic	No follow-up
Ahlbom, H. E.: Acta radiol., 1935, supp. 23, p. 1	F	59	4 mo.	Hard palate	1.5 × 2 cm.	Firm	No data	.....	Characteristic	No recurrence in 3 yr. 8 mo.
Skorpil, F.: Virchows Arch. f. path. Anat. **306**: 714, 1940	F	83	No data	Parotid	Child's fist	Nodular	No data	None	Characteristic	Recurrence after 2 yr.; no subsequent follow-up
Stöhr and Risak [5]	F	85	7 mo.	Left parotid	Child's fist	No data	No data	None	Called a tubular adenoma	Patient died after 8 yr.; no evidence of recurrence
Stöhr and Risak [5]	F	56	8 yr.	Right parotid	Size of apple	No data	Grayish red	No data	Characteristic	No follow-up
Ackerman, L. V.: Arch. Path. **36**: ?, 1943	M	76	6 mo.	Left parotid	2 cm. in diameter	Firm	Brownish red	None	Characteristic	No recurrence in 8 mo.

the age of 60 they are invariably present. McFarland's [4] first tumor of this type recurred after twelve years, and one reported by Stöhr [5] recurred two years after removal. Gruenfeld [6] pointed out that the origin of oncocytoma is often multicentric, and therefore both of these "recurrences" might have been due to the development of new tumors or to recurrence after incomplete removal of the primary tumors. Consequently, removal should be fairly radical with inclusion of practically all of the salivary gland. Metastases have never been reported. The tumors grow slowly, never reach a large size, are never attached to skin or bone and usually feel quite firm. On section they have either no capsule or an incomplete connective tissue capsule and are well delineated. Their color is either grayish or brownish red. Microscopically, they resemble the one reported here in practically all details. Ducts are rare within the tumor, but the tumor may arise from a transition of duct epithelium to oncocytes. The golden yellow granules present in the cytoplasm with the Masson trichrome stain may represent degenerative changes, but no definite conclusions are warranted. The appearance of these tumors is characteristic, and once seen they are easily recognized.

The term "oncocytoma" should be restricted to tumors of this type located in the salivary glands and should not be applied to those diagnosed as adenolymphoma,[7] as was done by Jaffé.[8] Numerous other examples of adenoma of the salivary glands have been described in the literature but, as McFarland [3] stated in 1927, "There can be little doubt that nearly all if not indeed all of the described adenomas are but mixed tumors of the salivary glands."

SUMMARY

Oncocytoma of the salivary glands is a rare tumor occurring in aged persons, with a slow clinical course, without metastasis and with a characteristic gross and microscopic appearance.

4. McFarland, J.: Am. J. M. Sc. **203**:502, 1942.
5. Stöhr, F., and Risak, E.: Arch. f. klin. Chir. **143**:609, 1926.
6. Gruenfeld, G. E., and Jorstad, L. H.: Am. J. Cancer **26**:571, 1936.
7. (*a*) Steinhardt, G.: Virchows Arch. f. path. Anat. **289**:624,1933. (*b*) Stout, A. P.: Personal communication to the author.
8. Jaffé, R. H.: Am. J. Cancer **16**:1415, 1932.

General Reviews

INFLUENCE OF THE ENDOCRINE GLANDS ON GROWTH AND AGING OF THE SKELETON

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HISTORICAL REVIEW

Disturbances of skeletal growth have been mentioned in myths and in early records of human history. The old Romans already distinguished between the *nanus*, a natural dwarf, and the *pumilo*, or unnatural dwarf, as they designated them. They thus may have recognized a difference between congenital dwarfism and the disproportionate dwarfism acquired during life. The tall, long-legged stature of castrates was fairly well known to the old physicians. However, only since the latter half of the past century have the origin and the nature of abnormal skeletal growth been given more attention.

With the advent and progress of endocrinology, certain disturbances of growth were recognized as being associated with endocrine dysfunctions. The epiphysiodiaphysial union was seen to be delayed in castrates and in certain dwarfs. Retarded skeletal growth was observed in patients suffering from hypothyroidism, hypopituitarism and diabetes. On the other hand, local overgrowth and general gigantism were noted in patients in whom the hypophysis was enlarged. More recent studies [1] of the skull of John Hunter's giant O'Brien revealed an enlargement of the sella turcica, and many cases of hyperplasia of the hypophysis associated with gigantism have been made known since.

From the Department of Pathology, New York University College of Medicine.

Delivered in shortened form as the Eleventh Walter M. Brickner Lecture at the Hospital for Joint Diseases, New York, Feb. 26, 1943.

1. Keith, A.: Lancet **1**:993, 1911.

Reports on the disease classified today as acromegaly date back to 1772.[2] Verga [3] in 1864, as well as Fritsche and Klebs [4] in 1884, suspected a relationship between this peculiar disorder and the ductless glands. In 1886 Marie [5] fully described the malady without, however, attributing it to any particular etiologic factor. Because eosinophilic adenoma of the anterior lobe of the hypophysis was found in many cases of acromegaly, most investigators of the past forty years have considered hyperpituitarism, and specifically hyperactivity of the acidophilic cells of the anterior lobe of the hypophysis, as the cause of the disease.[6] Marie himself even as late as 1911 did not express a definite view concerning this matter and wondered "if the solution does not lie in a somewhat different direction; that is to say, that the important fact concerns itself less, perhaps, with the quantitative function of the hypophysis than with the modification and alterations of its secretions." [7]

These early observations laid the basis for systematic studies on the influence of the endocrine glands on skeletal growth. Data concerning changes in body size and body weight occurring in various endocrine disorders were established. The effect of the ductless glands on the biochemistry and metabolism of the skeleton was analyzed, whereas histologic investigations were less frequently undertaken.

COMPARATIVE PATHOLOGY

Experimentally, the problem was approached as follows: (1) by extirpation of ductless glands, (2) by transplantation of endocrine glands or administration of hormonal substances to animals which had undergone removal of ductless glands previous to the treatment, (3) by transplantation of ductless glands and administration of hormonal preparations to normal animals.

Poncet [8] and Sellheim [9] were the first to demonstrate experimentally the delaying effect of castration on epiphysiodiaphysial union, and von Eiselsberg [10] obtained stunting in goats subsequent to removal of the thyroid gland. The systematic experiments of Gudernatsch,[11] Smith,[12] Allen,[13] Uhlenhuth [14] and others in larvae

2. (*a*) Davidoff, L. M.: Endocrinology **10**:453, 1926. (*b*) Baumgartner, L.: Bull. Hist. Med. **8**:446, 1940.

3. Verga, A.: R. Ist. Lomb. di sc. e lett. Rendic. Cl. di sc. matemat. e nat. **1**:111, 1864.

4. Fritsche and Klebs, E.: Beitrag zur Pathologie des Riesenwuchses, Leipzig, F. C. W. Vogel, 1884.

5. Marie, P.: Rev. de méd. **6**:297, 1886.

6. (*a*) Benda, C.: Berl. klin. Wchnschr. **37**:1205, 1900. (*b*) Bailey, P., and Davidoff, L. M.: Am. J. Path. **1**:185, 1925. (*c*) Bailey, P., and Cushing, H.: ibid. **4**:545, 1928.

7. Davidoff,[2a] p. 455.

8. Poncet, A.: (*a*) Mem. et compt.-rend. Soc. d. sc. méd. de Lyon **17**:178, 1878; (*b*) Compt. rend. Soc. de biol. **55**:65, 1903.

9. Sellheim, H.: (*a*) Beitr. z. Geburtsh. u. Gynäk. **2**:236, 1899; (*b*) Ztschr. f. Geburtsh. u. Gynäk. **74**:362, 1913.

10. von Eiselsberg, A.: Arch. f. klin. Chir. **49**:207, 1894.

11. Gudernatsch, J. F.: (*a*) Am. J. Anat. **15**:431, 1913; (*b*) Anat. Rec. **11**:357, 1916-1917.

12. (*a*) Smith, P. E.: Science **44**:280, 1916; (*b*) Anat. Rec. **11**:57, 1916; (*c*) The Pigmentary, Growth and Endocrine Disturbances Induced in the Anuran Tadpole by the Early Ablation of the Pars Buccalis of the Hypophysis, Anatomical Memoir 11, Philadelphia, Wistar Institute of Anatomy and Biology, 1920. (*d*) Smith, P. E., and Smith, I.: Endocrinology **7**:579, 1923.

13. Allen, B. M.: (*a*) Science **44**:755, 1916; (*b*) Biol. Bull. **32**:117, 1917; (*c*) J. Exper. Zool. **24**:499, 1918; (*d*) Anat. Rec. **14**:86, 1918; (*e*) Science **52**:274, 1920; (*f*) Anat. Rec. **20**:192, 1921; (*g*) J. Exper. Zool. **42**:13, 1923.

14. Uhlenhuth, E.: (*a*) J. Gen. Physiol. **1**:305 and 473, 1918-1919; (*b*) J. Exper. Zool. **25**:135, 1919; (*c*) Anat. Rec. **23**:43, 1922.

of cold-blooded animals demonstrated in a more exact way the effects of the hypophysis, the thyroid gland and the thymus on the growth and metamorphosis of anuran and urodele larvae.

In these larvae removal of the thyroid gland inhibited metamorphosis,[15] whereas growth in length continued and the larvae even reached an abnormally large size.[16] Administration of thyroid substances, on the other hand, accelerated the development of the larvae into the adult form, but body growth was inhibited.[17] Removal of the anterior lobe of the hypophysis inhibited both growth and metamorphosis of the larvae.[18] The adverse effects of removal of the thyroid gland on metamorphosis and growth of the larvae could be prevented or decreased by administration of thyroid substance[19]; similarly, hypophysectomy was at least partly counteracted by treatment with anterior hypophysial substance.[20] However, the action of anterior hypophysial substance in normal larvae soon became a matter of controversy: Actual overgrowth,[21] no definite influence on body growth[22] and even depression of growth[23] were observed in frogs, salamanders and axolotls.

In various growing mammals arrest or inhibition of growth was found not only subsequent to removal of either the thyroid or the pituitary gland but also after extirpation of the thymus and the adrenal glands. The main work, however, was focused on the effects of the thyroid gland and the anterior lobe of the hypophysial gland.

Robertson[24] in 1916 observed an increase over the normal in the body weights of growing mice after injection of an anterior hypophysial extract. Evans and Long[25] in 1921 obtained similar results and in addition increased body size in rats thus treated. Cushing and co-workers[26] found enlargement of internal organs and increased body size in dogs treated with anterior hypophysial extract. These experiments, as well as observations of pituitary dwarfism, on one hand, and of pituitary gigantism and acromegaly, on the other, furnished the basis for the theory of the existence of a specific hypophysial growth hormone. The growth-promoting effects of other endocrine glands, particularly the thyroid gland, were considered secondary inasmuch as these glands should affect the hypophysis and its output of growth hormone.

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15. (*a*) Schulze, W.: Arch. f. Entwcklngsmechn. d. Organ. **52**:232, 1922-1923. (*b*) Allen.[13c]

16. Hoskins, E. R., and Hoskins, M. M.: J. Exper. Zool. **29**:1, 1919. Uhlenhuth.[14a] Schulze.[15a]

17. Romeis, B.: Ztschr. f. d. ges. exper. Med. **4**:379, 1916. Swingle, W. W.: J. Exper. Zool. **24**:521, 1918. Gudernatsch.[11b]

18. Adler, L.: Arch. f. Entwcklngsmechn. d. Organ. **39**:21, 1914. Smith (footnote 12 *a* and *b*). Allen (footnote 13 *b*, *d* and *g*).

19. Jensen, C. O.: Compt. rend. Soc. de biol. **85**:391, 1921. Schulze.[15a]

20. Adams, A. E., and Gray, B.: Anat. Rec. **65**:69, 1936. Etkin, W.: J. Exper. Zool. **77**:347, 1938. Smith (footnote 12 *c* and *d*). Allen.[13f]

21. Clements, D. I., and Howes, N. H.: J. Exper. Biol. **15**:447 and 541, 1938. Swingle, W. W.: Anat. Rec. **20**:195, 1920-1921. Allen.[13e] Uhlenhuth.[14c]

22. Burns, R. K., Jr., and Buyse, A.: Anat. Rec. **51**:155, 1931. Burns, R. K., Jr.: ibid. **58**:415, 1934. Gardner, L. W.: ibid. **78**:139, 1940.

23. Blount, R. F.: Proc. Nat. Acad. Sc. **16**:218, 1930; J. Exper. Zool. **70**:132, 1935.

24. Robertson, T. B.: J. Biol. Chem. **24**:385, 1916.

25. Evans, H. M., and Long, J. A.: Anat. Rec. **21**:62, 1921; **23**:19, 1922; Proc. Nat. Acad. Sc. **8**:38, 1922.

26. Putnam, T. J.; Teel, H. M., and Benedict, E. B.: J. Physiol. **84**:157, 1928. Teel, H. M., and Cushing, H.: Endocrinology **14**:157, 1928.

NORMAL COURSE OF SKELETAL GROWTH AND AGING (SKELETAL TIME CURVE)

Growth of cartilage manifests itself in proliferation and hypertrophy of the cartilage cells.[27] Aging of cartilage is characterized by a decrease of its proliferative power and by regressive changes.[28] The latter consist of atrophy, hyalinization, disintegration and calcification of the cells. The matrix may become calcified and hyalinized or looser in texture; it may undergo fibrillation, mucoid softening or liquefaction. Both types of age changes may be present at the same time in different areas. In the bones age changes are indicated by predominance of destruction [29] over formation of osseous tissue.

Structural alterations in skeletal tissues go hand in hand with biochemical changes. Young cartilage contains abundant fat,[30] cholesterol [31] and phosphatase.[32] The amounts of fat and cholesterol decrease as the cartilage undergoes hypertrophy and aging. Growing bone contains more water and less inorganic material than old bone.[33]

During endochondral ossification the growth and aging of cartilage and bone go hand in hand.[34] New cartilage is formed from a mesenchymal layer of germinal cells. The cartilage cells assume columnar arrangement, and at a certain developmental stage they undergo hypertrophy. The hypertrophic cartilage cells calcify, are destroyed by advancing bone marrow and are replaced by the primary trabeculae. The latter are subsequently resorbed by bone marrow of the expanding diaphysial cavity.

The growth zones remain open and the cellular pattern is regular as long as growth and aging of cartilage and replacement by bone proceed at an even rate. The growth zones become narrow and close if growth cartilage decreases or if aging of cartilage and replacement by bone increase. These processes may result in stunting. Conversely, the epiphysial disks become enlarged if growth of cartilage predominates over the aging processes as a result either of intensified growth or of decreased aging or ossification of the cartilage.

Three main phases of skeletal growth and aging can be distinguished histologically during the life span of the mouse.[27b,c] During the first phase proliferation of the epiphysial cartilage predominates. This period is characterized by growth of bone in a lengthwise direction. Toward the end of this phase and coinciding with the development of full sexual maturity,[35] proliferation of cartilage decreases and the epiphysial plates narrow gradually. During the second phase, regressive changes of the cartilage are prominent, while ossification proceeds. The zones of growth continue to become narrower, and while there is no further increase in

27. (*a*) Dodds, G. S.: Anat. Rec. **46**:385, 1930. (*b*) Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941; (*c*) Endocrinology **29**:475, 1941; **31**:410, 1942. (*d*) Ingalls, T. H.: ibid. **29**:710, 1941.

28. Lang, F. J.: Virchows Arch. f. path. Anat. **256**:189, 1925. Boehmig, R.: Beitr. z. path. Anat. u. z. allg. Path. **81**:172, 1928. Amprino, R., and Bairati, A.: Ztschr. f. Zellforsch. u. mikr. Anat. **20**:143, 1933. Dodds.[27a] Silberberg and Silberberg (footnote 27 *b* and *c*). Ingalls.[27d]

29. Jaffe, H. L.: Arch. Surg. **20**:355, 1930. Jaffe, H. L., and Bodansky, A.: Arch. Path. **14**:432, 1932.

30. Okuyama, Y.: Mitt. a. d. Path. Inst. d. Med. Fak. Niigata **17**:1, 1930.

31. Bürger, M.: München. med. Wchnschr. **73**:1257, 1926.

32. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

33. Hammett, F. S.: J. Biol. Chem. **64**:409, 1923. Outhouse, J., and Mendel, L. B.: J. Exper. Zool. **64**:257, 1933.

34. Ruth, E. B.: Anat. Rec. **64**:1, 1935. Silberberg and Silberberg (footnote 27 *b* and *c*).

35. Green, C. V., and Fekete, E.: J. Exper. Zool. **66**:351, 1933.

the length of the bones, their shafts continue to grow in thickness owing to periosteal ossification. During the third phase, regression of such cartilage as is left proceeds, but in addition resorption of cartilage and bone becomes now the characteristic feature. The cortex of the long bones becomes thinned out, and the markedly narrowed epiphysial disks are perforated. However, under normal conditions complete epiphysiodiaphysial union does not occur in the mouse [27b,c] or in the rat.[36]

These three phases of skeletal growth and aging represent component parts of the skeletal time curve.[27c] The skeleton thus has its own peculiar time curve, as has been observed by Loeb [37] for tissues and organs in general. According to Loeb,[37] this time curve varies in different inbred strains and is genetically determined but can be modified to a certain extent by the action of hormones. The three phases of the skeletal time curve may overlap.

While the epiphysial cartilage ceases to grow as soon as the adult stage has been reached, the articular cartilage retains a certain capacity of growth throughout life.[38] In the joints changes of varying degree take place with advancing age [27b,c]: Slight alterations are characterized by some hyperplasia and hypertrophy of the cartilage with loosening of the matrix; more marked lesions consist of a moderate degree of both growth and regression of cartilage, while with the more severe changes intensified proliferation, hypertrophy and regression of cartilage and bone are seen. These lesions are comparable to those noted in the joints of aging human beings and may be classified with osteoarthritis.[39]

FACTORS MODIFYING THE NORMAL SKELETAL TIME CURVE

The duration of the skeletal time curve varies considerably in different strains of mice [40]: Strains C57 and CBA, for instance, exhibit a comparatively slow rate of skeletal growth and aging; conversely, the skeletal growth and aging of mice of strains D and C_3H proceeds more rapidly. In strain C57 the growth period lasts until the end of the fourth month of life; in strain D it is terminated as early as the age of 8 to 10 weeks. In strain C57 the second phase lasts until about the end of the first year of life, when the third phase sets in, initiating epiphysiodiaphysial union. In strain D, beginning perforations in the growth zones are seen as early as the age of 6 months. Moreover, strain D and strain C_3H differ from strains C57 and CBA in that they show deposition of larger amounts of bone.[27b, c] Similar strain differences have been observed not only in mice but also in guinea pigs.[41] The incidence and the severity of the articular age changes at a given age are greater in the rapidly aging strains than in the more slowly aging ones.[27b,c]

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36. Dawson, A. B.: Anat. Rec. **60**:501, 1934.

37. Loeb, L., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1940, vol. 36, p. 228.

38. Bennett, G. A., and Bauer, W.: J. Bone & Joint Surg. **17**:141, 1935. Silberberg and Silberberg (footnote 27 *b* and *c*).

39. Todd, T. W.: Am. J. Phys. Anthropol. **3**:283, 1920; Anat. Rec. **18**:264, 1920. Willis, T. A.: J. Bone & Joint Surg. **6**:316, 1924. Parker, F.; Keefer, C. S.; Myers, W. K., and Irwin, R. L.: Arch. Path. **17**:516, 1934. Keefer, C. S.; Parker, F.; Myers, W. K., and Irwin, R. L.: Arch. Int. Med. **53**:325, 1934. Bauer, W., and Bennett, G. A.: J. Bone & Joint Surg. **18**:1, 1936.

40. Howard, A.: Cancer Research **1**:503, 1941. Silberberg and Silberberg (footnote 27 *b* and *c*).

41. Eaton, O. N.: Am. J. Anat. **64**:485, 1939.

Skeletal development and aging proceed at a faster rate in females than in males [42] and in breeders more rapidly than in virgins.[43] The difference in skeletal aging of virgins and breeders may be indicative of hormonal influences, and comparable hormonal effects may be held responsible for the sex differences in skeletal aging in general. However, there is also a difference in skeletal aging between castrated females and castrated males [44] as an additional indication that the skeletal time curve is primarily genetically determined.

Findings in the human race agree with these observations in animals. Sex differences in the skeletal development of boys and girls have been established, girls exhibiting a faster rate of ossification than boys.[45] These differences become apparent before the onset of sexual maturity and even during fetal life [46]; they must, therefore, be attributed to genetic rather than to hormonal factors. At puberty, however, the differences become more accentuated, and the progress of skeletal aging in boys parallels more completely the development of secondary sex characteristics than the chronologic age. These observations may indicate that hormonal influences account at least partly for skeletal changes occurring after sexual maturity.

INFLUENCE OF ENDOCRINE GLANDS ON SKELETAL GROWTH AND AGING

Effects of the Anterior Lobe of the Hypophysis.—(*a*) Hypophysectomy: Grossly, in puppies,[47] growing rats [48] and ferrets,[49] removal of the anterior lobe of the hypophysis causes decrease or retardation but no cessation of skeletal growth. After extirpation of the anterior lobe growth may go on for several weeks or months, after which period it comes to a premature standstill.[50] The epiphysial plates of the long bones remain open for a considerable length of time and retain their juvenile characteristics.[51] In dogs the deciduous teeth persist [47c]; in rats the eruption of incisor and molar teeth is retarded and finally the growth and development of the teeth cease altogether.[52] The younger the animal at the beginning of the operation the more accentuated is the ensuing disturbance of

42. Spark, C. A., and Dawson, A. B.: Am. J. Anat. **41**:411, 1928. Latimer, H. B.: Anat. Rec. **40**:1, 1927. Silberberg and Silberberg (footnote *27 b* and *c*).

43. Slonaker, J. R.: Am. J. Physiol. **82**:318, 1927. Cole, H. H., and Hart, G. H.: Am. J. Physiol. **123**:589, 1938. Bogart, R.; Sperling, G.; Barnes, L. L., and Asdell, S. A.: Am. J. Physiol. **128**:355, 1940. Silberberg and Silberberg (footnote *27 b* and *c*).

44. (*a*) Hatai, S.: J. Exper. Zool. **18**:1, 1915. (*b*) Moore, C. R.: Biol. Bull. **43**:285, 1922. (*c*) Tang, Y. Z.: Anat. Rec. **80**:137, 1941.

45. Pryor, J. W.: Anat. Rec. **25**:257, 1923. Francis, C. C.: Am. J. Phys. Anthropol. **25**:323, 1939; Am. J. Dis. Child. **57**:817, 1939; Am. J. Phys. Anthropol. **27**:817, 1939. Bunak, V.: ibid. **26**:69, 1940. Greulich, W. W.: ibid. **27**:13, 1940; Endocrinology **30**:1023, 1942.

46. Hill, A. H.: Am. J. Phys. Anthropol. **24**:251, 1939.

47. (*a*) Aschner, B.: Arch. f. d. ges. Physiol. **146**:1, 1912. (*b*) Dott, N. M.: Quart. J. Exper. Physiol. **13**:241, 1923. (*c*) Reichert, F. L.: Proc. Soc. Exper. Biol. & Med. **25**:204 and 709, 1928. (*d*) Dandy, W. E., and Reichert, F. L.: Bull. Johns Hopkins Hosp. **62**:122, 1938. (*e*) Coryn, G.: Zentralbl. f. allg. Path. u. path. Anat. **73**:301, 1939; (*f*) Ann. d'anat. path. **16**:27, 1939.

48. (*a*) Mortimer, H.: Radiology **28**:5, 1937. (*b*) Freud, J., and Levie, L. H.: Arch. internat. de pharmacodyn. et de thérap. **59**:232, 1938. (*c*) Freud, J.; Levie, L. H., and Kroon, D. B.: J. Endocrinol. **1**:56, 1939. (*d*) Ray, R. D.; Evans, H. M., and Becks, H.: Am. J. Path. **17**: 509, 1941. (*e*) Ingalls, T. H., and Hayes, D. R.: Endocrinology **29**:720, 1941. (*f*) Van Eck, W. F., and Freud, J.: Acta brev. Neerland. **11**:43, 1941.

49. Parkes, A. S., and Rowlands, I. W.: Proc. Roy. Soc., London, s.B **125**:214, 1938.

50. Dandy and Reichert.[47d] Mortimer.[48a] Ingalls and Hayes.[48e]

51. Reichert.[47c] Mortimer.[48a]

52. (*a*) Schour, I., and Van Dyke, H. B.: J. Dent. Research **11**:873, 1931; (*b*) **14**:69, 1934. (*c*) Mortimer.[48a]

growth and development. Among ferrets [49] females respond more rapidly to hypophysectomy than males.

Histologically, the growth zones are narrowed and poor in cells.[53] The orientation of the cartilage columns is lacking, and the hypertrophy of the cartilage cells is decreased. The cartilage undergoes atrophy, hyalinization, calcification and liquefaction. The vascularization of the metaphysis is diminished. The trabeculae show deficient ossification and incomplete molding. The cessation of growth is further indicated by the appearance of a transverse bony plate underneath the epiphysial cartilage.[54] These changes resemble in some respects those seen in young undernourished animals.[55]

The latter similarity is probably not accidental. In hypophysectomized rats there are loss of appetite and decreased intake of food; the retention of nitrogen as well as the production of hepatic arginase are diminished [56]; however, the decreased consumption of food is apparently not primarily responsible for the metabolic disturbances: Hypophysectomized rats received by stomach tube a diet sufficient to permit body growth in normal animals.[57] In these rats the metabolic changes were similar to those seen in hypophysectomized animals that had taken food ad libitum: Less protein is utilized for anabolic processes, and there is decreased storage of nitrogen in both groups of animals.

(*b*) Hypopituitarism: Decreased skeletal growth associated with hypopituitarism has been reported in man,[58] hypoplasia or atrophy of other endocrine glands being present in most cases.

Hereditary dwarfism occurs in certain strains of mice.[59] The dwarfs show deficiency of eosinophils in the hypophysis and simultaneous atrophy of the thyroid and adrenal glands.[60] Skeletal growth and aging are pronouncedly delayed or inhibited in these.[61] During the growth period the bone age is far behind that of animals with hypophysis intact. However, the dwarf mice continue to grow at an age when normal animals have long ceased to grow; they may thus catch up to normal mice. These findings in the dwarfed mice may be interpreted as a delay of the skeletal time curve.

(*c*) Administration of Anterior Hypophysial Substance to Hypopituitary Animals: There is divergence of opinion concerning the effectiveness of anterior hypophysial preparations administered to animals which had undergone hypophysectomy previous to the treatment. In the hypophysectomized dog. Coryn [47e, f] was unable to restore growth by administration of anterior hypophysial substance. In the hypophysectomized rat, Mortimer [48a] noted a limited effect: There was a temporary resumption of the growth of the skull, but this came to a standstill after thirty to forty days' administration of the hypophysial preparation. Smith [62]

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53. Dott.[47b] Coryn.[47e] Freud and Levie.[48b] Freud, Levie and Kroon.[48c] Ray and others.[48d]

54. Silberberg and Silberberg. (footnote 27 *b* and *c*). Ray and others.[48d]

55. Silberberg, M., and Silberberg, R.: Arch. Path. **30**:675, 1940.

56. Lee, M. O., and Schaffer, N. K.: J. Nutrition **7**:337, 1934. Lee, M. O., and Ayres, G. B.: Endocrinology **20**:489, 1936. Marx, W.; Simpson, M. E.; Reinhardt, W. O., and Evans, H. M.: Am. J. Physiol. **135**:614, 1942.

57. Samuels, L. T.; Reineke, R. M., and Bauman, K. L.: Endocrinology **33**:87, 1943.

58. Paltauf, A.: Ueber den Zwergwuchs in anatomischer und gerichtsärztlicher Beziehung, nebst Bemerkungen über verwandte Wachsthumsstörungen des menschlichen Skeletes, Vienna, A. Hölder, 1891. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **62**:1, 1916.

59. Dawson, A. B.: Anat. Rec. **61**:485, 1934-1935.

60. Smith, P. E., and MacDowell, E. C.: Anat. Rec. **46**:249, 1930; **50**:85, 1931.

61. Boettiger, E., and Osborn, C. M.: Endocrinology **22**:447, 1938. Dawson.[59]

62. Smith, P. E.: Proc. Soc. Exper. Biol. & Med. **30**:1152, 1933.

found in hypophysectomized rats treated with anterior hypophysial substance only some stimulation of the proliferation of cartilage. On the other hand, Evans and his collaborators [48d] reported resumption of the growth of the cartilage and restoration of endochondral and periosteal ossification in hypophysectomized rats treated with anterior hypophysial substance, and Schour and Van Dyke [52b] observed restoration of the growth of the teeth under these conditions. Ultimately, Reichert [47c] and Freud and his co-workers [48c] recorded the proliferation of epiphysial cartilage, and the growth of the long bones increased even beyond that seen in normal rats with hypophysis intact.

In dwarf mice treatment with the lactogenic fraction of the anterior lobe of the hypophysis [63] or transplantation of the anterior lobe of the hypophysis [64] stimulates or restores skeletal growth to normal. Similarly, undersized hypopituitary children given injections of anterior hypophysial preparations [65] showed a temporary growth spurt. However, the stimulation of growth was only temporary, and the patients remained short adults. Comparable results were obtained by the administration of an extract containing the gonadotropic hormone of the anterior lobe of the hypophysis and, incidentally, with chorionic gonadotropin.[66]

(*d*) Hyperpituitarism: Studies on the effect of anterior hypophysial substance administered to animals with hypophysis intact have yielded divergent results which may be classified in four groups: (1) There was only insignificant stimulation of skeletal growth or none [67]; (2) the lengthwise growth of the bones was increased, leading in some instances to gigantism [68]; (3) the growth of the long bones was decreased or arrested [69]; (4) skeletal growth was accelerated but not absolutely increased,[70] and the final stature remained within the usual limits. Moreover, developmental processes, such as shedding of the deciduous teeth and eruption of the permanent ones, were hastened.[70a]

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63. Bates, R. W.; Laanes, T., and Riddle, O.: Proc. Soc. Exper. Biol. & Med. **33**:446, 1935. Bates, R. W.; Laanes, T.; MacDowell, E. C., and Riddle, O.: Endocrinology **31**: 53, 1942.

64. Kemp, T.: Klin. Wchnschr. **13**:1854, 1934. Smith and MacDowell.[60]

65. (*a*) Engelbach, W., and McMahon, A.: Endocrinology **8**:1, 1924. (*b*) Engelbach, W.; Schaefer, R. L., and Brosius, W. L.: ibid. **17**:250, 1933. (*c*) Falta, W. and Högler, F.: Klin. Wchnschr. **9**:1807, 1930. (*d*) Schaefer, R. L.: Endocrinology **20**:64, 1936. (*e*) Shelton, E. K.: ibid. **30**:1000, 1942. (*f*) Finkler, R. S., and Cohn, G. M.: Arch. Pediat. **60**:362, 1943.

66. Lurie, L. A., and Hertzman, J.: J. Clin. Endocrinol. **1**:717, 1941. Shelton.[65e]

67. (*a*) Smith, C. S.: Am. J. Physiol. **65**:277, 1923. (*b*) Larson, E.; Bergeim, O.; Barber, D. I., and Fisher, N. F.: Endocrinology **13**:63, 1929. (*c*) Johnson, G. E., and Sayles, E. D.: Physiol. Zoöl. **2**:285, 1929. (*d*) Johnson, G. E., and Hill, R. T.: Endocrinology **14**:400, 1930. (*e*) Korenchevsky, V.: Biochem. J. **24**:383, 1930. (*f*) Handelsman, M. B., and Gordon, E. F.: J. Pharmacol. & Exper. Therap. **38**:349, 1930.

68. (*a*) Goetsch, E.: Bull. Johns Hopkins Hosp. **27**:29, 1916. (*b*) Evans, H. M., and Simpson, M. E.: Anat. Rec. **35**:36, 1927; (*c*) J. A. M. A. **91**:1337, 1928. (*d*) Rubinstein, H. S., and Kolodner, L. J.: Anat. Rec. **58**:107, 1934. (*e*) Ross, E. S., and McLean, F. C.: Endocrinology **26**:329, 1940. (*f*) Freud and Levie.[48b]

69. Schaefer, E. A.: Quart. J. Physiol. **5**:203, 1912. Wulzen, R.: Am. J. Physiol. **34**: 127, 1914. Spencer, J.; d'Amour, C. A., and Gustavson, R. G.: Proc. Soc. Exper. Biol. & Med. **28**:500, 1931.

70. (*a*) Downs, W. G., Jr.: Arch. Path. **12**:37, 1931. (*b*) Lucke, H., and Hückel, R.: Arch. f. exper. Path. u. Pharmakol. **169**:290, 1933. (*c*) Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **32**:1423, 1935; (*d*) **34**:333, 1936. (*e*) Silberberg, M., and Silberberg, R.: ibid. **36**:622, 1937; (*f*) Arch. Path. **26**:1208, 1938; (*g*) Am. J. Path. **15**:547, 1939; (*h*) Arch. Path. **29**:355, 1940; (*i*) Am. J. Path. **17**:189, 1941; (*j*) **18**:1141, 1942. (*k*) Copher, G. H., and Silberberg, M.: To be published. (*l*) Lustig, B., and Wachtel, H.: Compt. rend. Soc. de biol. **132**:243, 1939. (*m*) Dott.[47b]

The epiphysial, costal and articular cartilages of young dogs,[71] guinea pigs,[72] rats [73] and mice [70i,j] have been examined histologically. Under the influence of anterior hypophysial extracts the cartilage cells undergo enhancement of their mitotic proliferation and hypertrophy associated with or followed by accentuated degeneration and calcification. Endochondral ossification is accelerated, and premature closure of the growth zones ensues.[74] Therefore, the intensification of the growth processes is followed by a sharp decline and premature standstill of growth.

In growing guinea pigs [70e] and rats [73a] the cartilage of the chondro-osseous junction of the ribs shows hyperplastic and hypertrophic growth. These changes, too, are temporary, and at later experimental stages marked ossification and resorption lead to a removal of the excess cartilage.[70j]

In the joints of young guinea pigs [70d] and rats [70b] arthropathic changes occur. In the joints of mice the incidence and the severity of the articular age changes are increased, and they set in earlier than in untreated animals.[70i,j]

Consequently, anterior hypophysial extracts accelerate the various phases of skeletal growth and aging, but no growth can be enforced in animals that have reached the end of their growth period.[75]

According to some investigators,[76] the growth-promoting hormone of the anterior lobe of the hypophysis can be extracted by alkaline but not by acid solutions. However, the effects of anterior hypophysial substance are fundamentally the same whether a crude acid extract or an alkaline preparation (antuitrin G) is administered.[70j] The response depends on the preparation used, the species of animals to which the preparation is given and on the mode of application: Antuitrin G, for instance, elicits a more marked reaction in guinea pigs than in mice,[70j] and phyone is effective in rats if injected intraperitoneally but not if it is given subcutaneously.[68e]

Some authors reported resumption of growth of the inactive epiphysial cartilage in adult rats 6 months of age under the influence of anterior hypophysial substance.[77] However, the question can be raised, whether this represents a true revival of growth. Donaldson [78] and Strong [79] have shown that rats still grow at the age of 474 days and later. Therefore, the stimulation obtained in these rats subsequent to the treatment with anterior hypophysial substance might be comparable to the findings in mice [70i,j]; it may be that the remaining growth capacity of the cartilage is forced to exhaust itself more rapidly than it would under normal conditions.

In immature guinea pigs [70f] and young rabbits,[80] transplants of the anterior lobe of the bovine hypophysis likewise hasten and intensify the course of skeletal

71. Dott.[47b] Coryn.[47f] Copher and Silberberg.[70k]

72. Zuck, T. T.: Am. J. Phys. Anthropol. **23**:498, 1938. Silberberg (footnote 70 *c* and *d*). Silberberg and Silberberg (footnote 70 *e*, *g*, *h* and *j*).

73. (*a*) Ray, R. D.; Evans, H. M., and Becks, H.: Anat. Rec. **82**:67, 1942. (*b*) Freud and others. [48c] (*c*) Ray and others.[48d] (*d*) Lucke and Hückel.[70b]

74. Silberberg.[70c] Silberberg and Silberberg (footnote 70 *f* and *i*).

75. Silberberg and Silberberg.[27e] Silberberg and Silberberg.[70j]

76. Thomson, D. L., and Collip, J. B., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1940, vol. 2, p. 309. Freud, J.; Laqueur, E., and Mühlbock, O., in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 301. Ray and others.[48d] Ross and McLean.[68e]

77. Ross and McLean.[68e] Ray and others.[73a]

78. Donaldson, H. H., and Conroe, S. B.: Am. J. Anat. **26**:237, 1919.

79. Strong, R. M.: Am. J. Anat. **36**:313, 1926.

80. Pereira, S.: Lyon chir. **34**:673, 1937.

development and aging. The effects of these transplants are similar to those exerted by the extracts of the anterior lobe of the hypophysis.

By contrast, transplants of the anterior lobe of the hypophysis to brother and sister mice [70]—syngenesiotransplants—inhibit the proliferation of epiphysial cartilage, but in these animals the growth period lasts longer than ordinarily; in other words, the cartilage continues to grow, although at a decreased rate, at an age when normally its proliferation would have been terminated. In the metaphysis and shaft more bone is present than is observed normally or after administration of anterior hypophysial extracts. Regressive and resorptive processes in the cartilage set in later than ordinarily. But once they have begun, they progress with such rapidity and intensity that the onset of epiphysiodiaphysial union is hastened. Therefore, syngenesiotransplants of the anterior lobe of the hypophysis likewise in the end accelerate skeletal aging, but the underlying mechanism is different from that set in motion by the anterior hypophysial extract. The transplants exert a marked gonadotropic effect, and the difference between their action on bone growth and that of the extracts may perhaps be attributed to an increased output of estrogen in mice bearing the transplants.

The acceleration of skeletal aging caused by anterior hypophysial substance is also illustrated by the course of bone repair under the influence of this substance. In growing guinea pigs [81] and rabbits [82] the various phases of bone repair are hastened. In salamanders,[83] newts [83] and frogs [84] the regeneration of amputated tails is accelerated. The results seem, however, to depend on the phase of regeneration at which the administration of the anterior hypophysial substance is initiated. The hormone apparently has an accelerating effect on growth of formed structures rather than on the rate of regeneration of new ones.

Effects of the Thyroid Gland.—(*a*) Hypothyroidism and Athyroidism: Arrest or retardation of growth, deformities and faulty molding of the long and the flat bones have been recorded in athyreotic and in hypothyroid children,[85] and in thyroidectomized monkeys,[86] goats,[87] sheep,[88] dogs,[89] cats,[90] rabbits,[91] guinea pigs,[92] rats,[93] fowl,[94] lizards [95] and tadpoles.[96] Moreover, in monkeys,[97] the eruption of the teeth is retarded and the formation of dentin is incomplete.

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81. Silberberg, M., and Silberberg, R.: Proc. Soc. Exper. Biol. & Med. **33**:177, 1935.
82. Eitel, H., and Lexer, E. W.: Arch. f. klin. Chir. **185**:587, 1936.
83. Richardson, D.: (*a*) Anat. Rec. **55**:73, 1933; (*b*) J. Exper. Zool. **83**:407, 1940.
84. Herrell, W. E.: Anat. Rec. **59**:47, 1934. Puckett, W. O.: ibid. **71**:337, 1938.
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92. (*a*) Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **33**:554, 1936. (*b*) Silberberg, M., and Silberberg, R.: Am. J. Path. **16**:505, 1940.
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(Footnotes continued on next page)

In the long bones the growth deficiency manifests itself in the epiphysis and to a lesser extent in the shaft. Periosteal ossification is less affected than endochondral ossification. Consequently, the long bones are comparatively short and thick, and the epiphyses appear plump and flattened out. The growth zones remain open for an undue length of time, and the course of skeletal aging is thus retarded. The suppression of metamorphosis in tadpoles, whose growth in length continues subsequent to thyroidectomy,[96] may go hand in hand with a delay of skeletal aging.

The degree of the disturbance in skeletal growth and aging seen after removal of the thyroid gland depends on the species and on the age and the sex of the animal used for the experiment: Cats, guinea pigs and lizards react less readily than sheep, goats and dogs. Even within the same species, however, differences in responsiveness exist: Binswanger [89c] found growth decreased in only 7 of 13 thyroidectomized dogs. The depression of growth was most accentuated immediately after operation; in some instances it was temporary and was compensated later on. The inhibition of growth was the more pronounced the younger the dog was at the time of operation. This conclusion, however, does not seem to hold good in general. Hammett [93d,f-h,k] noted the retardation of growth to be more marked in rats operated on at the age of 100 days than in those operated on at the age of 75 days. Moreover, the inhibition of growth was more accentuated in females than in males.[93e, i-k]

Histologically, the main change consists in a delay of ossification, whereas the proliferation of the cartilage may be maintained or may be even temporarily increased. However, there are retardation or lack of transformation of columnar into hypertrophic cartilage cells, decreased calcification, inhibition of breakdown of the hypertrophic cartilage and of its replacement by bone. Vascular and cellular resorption are markedly decreased. Consequently, the primary trabeculae are incompletely ossified and persist longer than ordinarily.[98] The inhibition of resorptive processes may explain the general thickening of the bones and the faulty molding of epiphyses and jaws frequently seen in hypothyroidism.

Bone repair after fractures is delayed in thyroidectomized animals.[99] Similarly, regeneration of amputated limbs in larvae of triton is retarded.[100]

(*b*) Hyperthyroidism: The effects of thyroid hormone on skeletal growth and aging depend on the dose given and the duration of the experiment. In rats [93a] and pullets,[101] small doses of thyroxin do not influence skeletal growth. In dogs,[102] cats,[102] rabbits,[101] guinea pigs,[103a] rats [104] and mice [105] the skeletal growth rate is

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(*g*) Am. J. Physiol. **68**:1, 1924; (*h*) **70**:259, 1924; (*i*) Endocrinology **10**:29, 1926; (*j*) Am. J. Physiol. **77**:527, 1926; (*k*) Endocrinology **10**:145, 1926. (*l*) Flower, C. F., and Evans, H. M.: Anat. Rec. **29**:383, 1925. (*m*) Becks, H.; Kibrik, E. A., and Evans, H. M.: J. Exper. Zool. **89**:297, 1942. (*n*) Patras, M. C.; Templeton, R. D.; Ferguson, R. L., and Hummon, I. F.: Am. J. Physiol. **133**:617, 1941.

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increased subsequent to the administration of moderately high doses of the hormone. High doses of thyroxin, on the other hand, cause retardation or cessation of growth in rabbits,[101] guinea pigs,[106] rats,[107] mice,[103b] canaries [101] and fishes.[108] On the other hand, thyroid hormone may counteract gigantism in man.[109]

The divergence in the effects of large and small doses of thyroid hormone on the skeleton of higher species can be correlated to similar observations in lower organisms. In plants, as well as in unicellular animals,[110] small doses of thyroxin promote cell division and growth, whereas large doses inhibit these processes.

The aging effect of thyroid hormone on the skeleton manifests itself in premature epiphysiodiaphysial union in dogs [47b] and mice,[111] in accelerated eruption of teeth in newborn rats [112] and in increased deposition of dentin in the teeth of young monkeys.[97]

Growing individuals suffering from long-standing hyperthyroidism are often taller,[113] and children treated with thyroid hormone exhibit more advanced osseous development than normal adolescents of corresponding age.[114] Thus, linear growth is enhanced to a moderate degree only, whereas skeletal aging is markedly accentuated. Thyroid hormone administered to young hypothyroid individuals increased by about 600 to 900 per cent the previous annual increment; during the second year of treatment, however, the gain in weight was 25 to 30 per cent less than in the first year even if the dose of thyroid hormone had been increased twice or three times during the second year; during the third year of treatment the growth rate dropped to normal.[65e] These observations in children show, as do the experimental results, that it is not possible to stimulate the growth of the cartilage beyond certain limits.[27c]

Histologically in cats, rabbits, guinea pigs and rats thyroid hormone temporarily intensifies and hastens growth of the epiphysial cartilage [115]; the regressive and resorptive processes are even more accelerated and intensified than after administration of anterior hypophysial substance. Perforations of the growth zones and cessation of growth appear at an age when normally proliferation of cartilage is still in progress. Thus, stunting may result under the influence of high doses of thyroid hormone. The hormone likewise hastens the regeneration of amputated tails of amphibian larvae.[116]

In the articular cartilage thyroxin increases the sclerosis and hyalinization of the ground substance.[103b] This effect seems to counteract the hypertrophy, swelling

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116. Speidel, C. C.: Am. J. Anat. **43**:103, 1929.

and liquefaction of the cartilage that lead to arthropathic changes in normal old animals. Thus, the severity and the incidence of these changes are decreased subsequent to the administration of thyroxin. Favorable effects of thyroxid hormone on the course of arthropathies have been noted in man, whereas arthritic lesions are not infrequently seen in persons with hypothyroidism.[117]

Effects of the Parathyroid Glands.—The skeletal tissue of various species differ in their response to parathyroid hormone. In growing dogs,[118a] rats [119] and axolotls [120] stunting has been observed subsequent to the administration of parathyroid extract. On the other hand, in cats [119a] and chickens [121] and also in some instances in rats [122] bone growth was not definitely altered. Finally, in dogs [123] and suckling rats,[124] overgrowth of cartilage with delayed ossification has been recorded. In part, these differences may be due to the dose of the extract given, the calcium supply and the age of the animal at the beginning of the treatment. The younger the animal and the higher the dose the more pronounced are the ensuing disturbances of growth.

Bone repair is delayed after parathyroidectomy.[125] However, the possibility cannot be excluded that in some cases at least the simultaneous removal of the thyroid gland may account for this retardation. On the other hand, callus formation is stimulated after administration of parathyroid extract in dogs,[125c] rats [126] and pigeons.[127]

Histologically, the effect of parathyroid hormone on the cartilage is similar to that exerted by thyroid hormone except that parathyroid hormone fails to stimulate the proliferation of the epiphysial cartilage.[128] It promotes the hypertrophy, calcification and disintegration of the growing cartilage and stimulates the formation of osteogenic tissue. If enough calcium is available, bone formation may be increased.[129] However, under certain experimental conditions [118a-c] the stimulation of the connective tissue may lead to resorption of bone. The latter changes resemble those seen in fibrous osteitis. Both increased resorption and increased deposition of bone may be reversed under the influence of prolonged administration of parathyroid extract.[130] Increased resorption of cartilage and bone may lead to acceleration of the onset of epiphysiodiaphysial union.[128]

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119. (*a*) Bauer, W.; Aub, J. C., and Albright, F.: J. Exper. Med. **49**:145, 1929. (*b*) Selye, H.: Arch. Path. **14**:60, 1932. (*c*) Burrows, R. B.: Am. J. Anat. **62**:237, 1938.

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129. (*a*) Selye, H.: Endocrinology **16**:547, 1932. (*b*) Bauer and others.[119a] (*c*) Silberberg and Silberberg.[128]

130. Burrows.[119c] Silberberg and Silberberg.[128] Selye.[129a]

Effects of Potassium Iodide.—Cameron and Carmichael [131] found no influence of sodium iodide on body length in rabbits and rats. According to some investigators,[132] growth was retarded in rats subsequent to the administration of potassium iodide, but no such effect was observable in mice.[133] Still other authors reported acceleration and increase of skeletal growth in pigs,[134] sheep,[135] chickens,[136] rats [137] and mice.[138] Children given iodine are taller than those kept on a normal standard diet.[139]

Histologically, in growing chickens,[136a] guinea pigs [140] and mice [141] potassium iodide stimulates the growth of the cartilage. Subsequently, regression, calcification, ossification and resorption of cartilage and bone are increased, and the onset of epiphysiodiaphysial union is hastened. Potassium iodide thus promotes skeletal growth as well as aging.

The substance is also effective in thyroidectomized animals.[142] It may therefore be concluded that potassium iodide acts at least partly without intermediation of the thyroid gland. However, potassium iodide stimulates the resorptive processes less in thyroidectomized animals than in animals with the thyroid gland intact. The latter effect seems thus to be contingent on the presence of the thyroid gland.

Effects of the Sex Glands.—(*a*) Castration: In male rats,[143] guinea pigs [144] and chickens [8b] growth is decreased during the first months following castration. The growth deficiency is subsequently compensated by intensified growth so that the ultimate size of the long bones is the same as in noncastrated animals. In the deer,[145] the antlers fail to grow, and in the boar,[8b] the incisor teeth fall out, if castration is performed early in life.

In spayed female rats,[146] guinea pigs,[144] dogs,[9b] rabbits [147] and cattle [147] skeletal growth is temporarily increased. Here again the changes are pronounced only

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142. Silberberg, M., and Silberberg, R.: Arch. Path. **28**:846, 1939.

143. (*a*) Stotsenburg, J. M.: Anat. Rec. **3**:233, 1909; (*b*) **7**:183, 1913. (*c*) Hatai, S.: J. Exper. Zool. **18**:1, 1915. (*d*) Van Wagenen, G.: Am. J. Physiol. **84**:461, 1928. (*e*) Commins, W. D.: J. Exper. Zool. **63**:573, 1932. (*f*) Lawless, I. I.: Anat. Rec. **66**:455, 1936. (*g*) Rubinstein, H. S.; Abarbanel, A. R., and Kurland, A. A.: Endocrinology **25**:397, 1939. (*h*) Tang.[44c]

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147. Shattock, S. G., and Seligmann, C. G.: Proc. Roy. Soc. Med. (Path. Sect.) **3**:102, 1909-1910.

during the first months following ovariectomy. At later stages the differences in skeletal size disappear, owing to a compensatory decrease of the growth rate.

Callus formation is at first decreased and subsequently increased in castrated animals.[148]

Indecisive or negative results obtained in various species[149] might be attributed to the fact that the animals had been killed at a time when the initial deviations of growth had been compensated.

Histologically, in growing castrated animals the epiphysial, costal and articular cartilages undergo increased hyperplasia and hypertrophy. In female guinea pigs calcification and ossification are not accelerated at the same rate.[150] Consequently, the onset and the progress of regressive and resorptive processes are retarded and epiphysial closure is delayed.[151] Thus the epiphysial cartilage retains its youthful character for an unusually long period. However, once aging of the cartilage has set in, it proceeds faster than ordinarily. This compensates for the delay in skeletal aging suffered during the preceding periods and may explain why gigantism does not occur in castrated animals. Owing to the predominance of resorption over formation of osseous substance, the bones remain slender and thinner than usual.

In the castrated male hyperplasia and regression of the cartilage preponderate, whereas in the castrated female hypertrophic changes are more accentuated. Furthermore, in the castrated male calcification and ossification of the cartilage outbalance proliferation, as is indicated by the narrow calcified zones of growth. The not uncommon fibrosis of the subepiphysial marrow surrounding the trabeculae may be due to rapid resorption of the primary spicules.[150b]

The sex differences in the response of the skeletal tissues might be correlated to the greater stimulating effect that castration exerts on the anterior lobe of the hypophysis and on the thyroid gland in males as compared with females.[152] The greater amounts of anterior hypophysial and thyroid hormones released in the castrated male may account for the intensification of resorption and ossification in the latter.

(*b*) Administration of Estrogen: In macaques,[153] guinea pigs,[154] rats[155] and cocks[155c] the growth of the long bones stops or is inhibited under prolonged administration of high doses of estrogen, and in macaques[153] certain milk teeth fail to be shed. The bones remain shorter than ordinarily; they are hard and brittle. Girls showing precocious sexual maturity have short legs and exhibit advanced bone age.[156] On the other hand, estrogen has been successfully used in curbing excessive growth in sexually underdeveloped girls.[157]

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150. Silberberg, M., and Silberberg, R.: (*a*) Proc. Soc. Exper. Biol. & Med. **37**:446, 1937; (*b*) Am. J. Path. **16**:491, 1940.

151. Poncet.[5b] Sellheim.[9a] Silberberg and Silberberg.[150]

152. Kippen, A. A., and Loeb, L.: Endocrinology **20**:201, 1936.

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154. Silberberg, M., and Silberberg, R.: (*a*) Arch. Path. **28**:340, 1939; (*b*) Am. J. Anat. **69**:295, 1941.

155. (*a*) Spencer, J.; Gustavson, R. G., and d'Amour, F. E.: Proc. Soc. Exper. Biol. & Med. **28**:500, 1931. (*b*) d'Amour, F. E.; Gustavson, R. G., and Spencer, J.: Am. J. Anat. **50**:129, 1932. (*c*) Zondek, B.: Lancet **2**:842, 1936; (*d*) **1**:1107, 1938; (*e*) Am. J. Cancer **33**:555, 1938. (*f*) Billeter, O. A.: Am. J. Anat. **60**:367, 1937. (*g*) Levie, L. H.: Acta brev. Neerland. **8**:53, 1938. (*h*) Noble, R. L.: Lancet **1**:192, 1938. (*i*) J. Endocrinol. **1**:216, 1939 (*j*) Silberberg and Silberberg.[154b]

156. Reilly, W. A.: Endocrinology **18**:117, 1934. Bayer, L. M.: ibid. **24**:260, 1939.

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In growing mice treated with estrogen [154b] there is no marked decrease of the length of the bones. However, their breaking strength is noticeably increased.[158] In bufo metamorphosis is accelerated but no influence on body growth is observable.[159]

Histologically, estrogen decreases the growth rate of the cartilage and hastens the onset and progress of regressive change, in particular hyalinization and calcification of the cartilage. An intensification of these processes leads to narrowing of the epiphysial disks and to intensified ossification.[160] Thus, the structural age of the cartilage is markedly advanced over the normal.[161] On the other hand, the progress of resorptive processes is inhibited by estrogen.[162] Therefore, in spite of the fact that skeletal aging is accelerated, perforations in the growth zones appear much later than usual, and the primary trabeculae persist longer than ordinarily. Consequently, the amount of bone is greater than normal. The expansion of the marrow cavity is inhibited, and instead of cellular marrow an interlaced trabecular bony network is formed. Moreover, in some species and strains of mice there may occur actual overproduction of bone in addition to the excess bone present as a result of decreased resorption.

According to Gardner and his co-workers,[163] this active osteoblastic endosteal bone formation is a primary effect of estrogen in mice as well as in various species of birds. In chickens, pigeons, sparrows and ducks the ovulatory cycle is accompanied by cyclic hyperossification and resorption [164] of the long bones. Under certain experimental conditions these changes can be reproduced by administration of estrogen or of a gonadotropic substance.[165] The significance of these factors has recently been reviewed by Gardner [164b] and McLean.[164c]

Under prolonged administration of estrogen the initial effects are reversed: Resorptive processes are resumed and then they proceed rapidly.[166] Perforations in the growth zones are initiated and the excess bone is removed frequently with simultaneous appearance of osteoid tissue.[167] Bremer [167a-d] correlated these effects of estrogen on the long bones with the changes seen during pneumatization of

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159. Moracci, E.: Arch. di ostet. e ginec. **3**:288, 1939.

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162. Day, H. G., and Follis, R. H., Jr.: Endocrinology **28**:83, 1941. Silberberg and Silberberg.[154]

163. (*a*) Pfeiffer, C. A., and Gardner, W. U.: Endocrinology **23**:485, 1938. (*b*) Kirschbaum, A.; Pfeiffer, C. A.; Van Heuverswyn, I., and Gardner, W. U.: Anat. Rec. **75**:249, 1939. (*c*) Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: Proc. Soc. Exper. Biol. & Med. **41**:80, 1939. (*d*) Pfeiffer, C. A.; Kirschbaum, A., and Gardner, W. U.: Yale J. Biol. & Med. **13**:279, 1940. (*e*) Gardner, W. U.: Anat. Rec. **76**:22, 1940. (*f*) Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Shaw, J. C.: Endocrinology **28**:458, 1941. (*g*) Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **38**:599, 1938. (*h*) Gardner, W. U.: Endocrinology **32**:149, 1943.

164. (*a*) Keyes, P., and Potter, T. S.: Anat. Rec. **60**:377, 1934. (*b*) Gardner, W. U.: Physiol. Rev. **21**:193, 1943. (*c*) McLean, F. C., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1943, vol. 5, p. 79. (*d*) Gardner and others (footnote 163 *a* to *h*).

165. Gardner and others (footnote 163 *a* to *h*). Gardner.[164b] McLean.[164c]

166. Simpson, M. E.; Kibrick, E. A.; Becks, H., and Evans, H. M.: Endocrinology **30**:286, 1942. Silberberg and Silberberg.[154] Day and Follis.[162]

167. (*a*) Bremer, J. L.: Anat. Rec. **76**:9, 1940; (*b*) Arch. Path. **32**:200, 1941; (*c*) Anat. Rec. **79**:11, 1941; (*d*) J. Bone & Joint Surg. **24**:32, 1942. (*e*) Miller, E. W.; Orr, J. W., and Pybus, F. C.: J. Path. & Bact. **55**:137, 1943. (*f*) Silberberg and Silberberg.[154]

avian bones. He suggests that estrogen exerts its effect on the skeleton through the intermediation of the parathyroid glands.[167b]

Guinea pigs,[154a] rats,[168] dogs[169] and sparrows[163b,d] are less responsive to estrogen than are pigeons,[170] chicken[164b] and ducks.[164b] However, even within the same species considerable strain differences occur. Mice of the slowly aging strains C57 and CBA react more readily and intensely than mice showing a fast rate of skeletal aging.[171] Similar strain differences have been established for ducks.[172]

Since estrogen intensifies the hyalinization of the cartilage, the incidence and the severity of the arthropathic age changes in the joints are decreased.[173] In women, likewise, arthritic lesions seen during the menopause have been favorably influenced by treatment with estrogen.[174]

(*c*) Administration of Progesterone: In growing guinea pigs treated with this hormone for two months the bones were thinner and more delicate than ordinarily[175]; their length, however, was the same as that in control animals. In rats no influence on the length of the bones was noted.[176]

Histologically, growth of cartilage is not affected or it is slightly stimulated, and the replacement of cartilage by bone lags behind the normal. The osseous tissues contain comparatively little ground substance and rather large osteocytes. The youthful characteristics of cartilage and bone are thus maintained for an unusually long period. The structure of the bones resembles that found in castrated animals. Consequently, progesterone prevents the aging effects of estrogen for a certain length of time; it presumably acts by neutralization of the estrogen. At later stages, however, the regressive changes in cartilage and bone proceed more rapidly than usual, and in the end the degree of skeletal aging in guinea pigs and mice does not differ from that found in untreated animals.[177]

(*d*) Administration of Androgen: According to some investigators large amounts of testosterone cause stunting in chickens[178] and rats.[179] According to others, however, growth is not inhibited even if high doses of testosterone are injected.[180] In newborn rats treated with testosterone the os penis and the epiphysis of the ischiadic tuberosities appear earlier than usual,[70l] and the bones of female mice show lesser breaking strength than those of the controls.[163g,h]

In man[181] testosterone hastens epiphysial maturation and aging; it also inhibits excessive growth in adolescent boys.[157] There are, however, indications that in the hypogenital or in the castrate individual testosterone may under certain con-

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168. Silberberg and Silberberg.[154b] Day and Follis.[162] Simpson and others.[166]

169. (*a*) Sutro, C. J.: Proc. Soc. Exper. Biol. & Med. **44**:151, 1940. (*b*) Sutro, C. J., and Pomerantz, L.: Arch. Path. **33**:305, 1942.

170. Landauer and others.[163f] Gardner and Pfeiffer.[162g] Gardner.[164b]

171. Silberberg and Silberberg.[154b] Sutro and Pomerantz.[169b]

172. Landauer and others.[163f] Gardner.[164b]

173. Silberberg and Silberberg.[27e] Silberberg and Silberberg.[154b]

174. Cawadias, A. P.: Brit. J. Rheumat. **1**:48, 1938. Osgood, R. B.: Am. J. M. Sc. **200**: 429, 1943. Pemberton, R., and Scull, C. W.: Ann. Int. Med. **19**:482, 1943.

175. Silberberg, M., and Silberberg, R.: Arch. Path. **31**:85, 1941.

176. Sosa Gallardo, J. B., and Arias, D.: Rev. Soc. argent. de biol. **17**:147, 1941.

177. Silberberg and Silberberg.[27e] Sosa Gallardo and Arias.[176]

178. Ettinger, G. H.: Anat. Rec. **82**:408, 1942.

179. Rubinstein, H. S.; Kurland, A. A., and Goodwin, M.: Endocrinology **25**:724, 1939.

180. (*a*) McEuen, C. S.; Selye, H., and Collip, J. B.: Proc. Soc. Exper. Biol. & Med. **36**:390, 1937. (*b*) Webster, B., and Hoskins, W.: ibid. **45**:72, 1940. (*c*) Turner, H. H.; Lachmann, E., and Hellbaum, A. A.: Endocrinology **29**:425, 1941.

181. McGullagh, E. P., and McGurl, F. J.: Endocrinology **26**:377, 1940. McGullagh, E. P., and Rossmiller, H. R.: J. Clin. Endocrinol. **1**:507, 1941.

ditions act as a growth stimulant. This has been shown in experiments [701] as well as in a number of boys treated for hypogenitalism.[182]

In these instances testosterone causes a sudden accentuation of growth, but this is followed by a steep drop in the growth rate and by early cessation of growth, even if treatment is continued.

The growth-promoting influence of testosterone can be increased by simultaneous administration of chorionic gonadotropin.[182a] The latter hormone also stimulates growth if injected alone. Little is as yet known about its mode of action; it possibly acts through the intermediation of the sex glands.

Histologically, testosterone propionate accelerates skeletal aging by promoting degeneration, hyalinization and calcification of the cartilage; it also inhibits proliferation and resorption of cartilage and bone. Thus, the effects of testosterone propionate are similar in kind, although milder in degree, to those exerted by estrogen. Under prolonged administration of testosterone propionate, the resorptive processes are resumed, the excessive amounts of bone are dissolved and epiphysiodiaphysial perforations may be initiated even sooner than ordinarily. Such hastening of skeletal aging is observable in mice [183] and rats.[180c]

Effects of the Thymus.—In thymectomized young rats retardation of growth has been noted by some investigators.[184] The inhibition of growth is more marked if thymectomy is repeated in successive generations. Other authors, however, have failed to observe any influence on growth under the same conditions.[185] There is also no agreement as to whether administration of thymus extract to thymectomized or normal animals accelerates skeletal development [186] or whether it has no effect at all on body growth.[187] Reports of histologic studies are not available.

Effects of the Adrenal Glands.—The data are scarce. In rats after removal of the adrenal glands stunting and arrest of growth occur.[48e] On the other hand, in hypophysectomized pigeons and mice, Riddle and his collaborators [188] found restoration of growth following treatment with adrenal cortex extract.

Histologically, Ingalls and Hayes [48e] observed in adrenalectomized rats decreased proliferation of the cartilage, deficient calcification and ossification and at later stages demineralization and atrophy of the bones. The changes are similar to those reported by these authors subsequent to hypophysectomy. They conclude, therefore, that the changes in the skeletal tissues after the operations are nonspecific.

INTERACTION OF SEVERAL ENDOCRINE GLANDS

Anterior Hyophysial and Thyroid Hormone.—These two hormones act as synergists. In hypophysectomized rats there is more complete restoration of

182. (*a*) Thompson, W. O.; Heckel, N. J., and Morris, J. P.: Endocrinology **30**:1042, 1942. (*b*) Finkler and Cohn.[65f] (*c*) Webster and Hoskins.[180b] (*d*) McGullagh and others.[181]

183. Silberberg, M., and Silberberg, R.: Arch. Path. **32**:85, 1941.

184. (*a*) Einhorn, N. H., and Rowntree, L. G.: Endocrinology **20**:342, 1936; (*b*) Science **83**:443, 1936; (*c*) Endocrinology **22**:335, 1938. (*d*) Rowntree, L. G.; Steinberg, A.; Einhorn, N. H., and Schaffer, N. K.: ibid. **23**:584, 1938. (*e*) Comsia, J.: Compt. rend. Soc. de biol. **127**:903, 1938.

185. Segaloff, A., and Nelson, W. O.: (*a*) Am. J. Physiol. **130**:671, 1940; (*b*) Endocrinology **29**:483, 1941.

186. Allardyce, J.; Dale, U.; Smith, D., and Griffin, P.: Endocrinology **27**:994, 1940. Einhorn and others (footnotes 184 *a*, *b*, *c* and *d*).

187. Burill, M. W., and Ivy, A. C.: Endocrinology **28**:94, 1941. Neuwirth, I., and Venokur, H. I.: Science **94**:233, 1941. Segaloff and Nelson.[185b]

188. Miller, R. A., and Riddle, O.: (*a*) Anat. Rec. **84**:490, 1942; (*b*) Endocrinology **32**: 463, 1943.

growth if thyroid hormone is administered in addition to anterior hypophysial substance.[189] In rats [189b] as well as in growing mice and guinea pigs [27c] with the hypophysis intact the growth-promoting and aging effect of anterior hypophysial substance are intensified by simultaneous administration of thyroxin. The proliferation and the ossification of the epiphysial cartilage are more pronounced than after treatment with anterior hypophysial substance alone. Subsequently, the resorptive processes are so markedly intensified that they soon outbalance the bone-forming effect of the anterior hypophysial substance. Thus, perforations in the growth zones appear earlier than after treatment with either substance alone.

The anterior hypophysial substance exerts its influence also in growing animals which have undergone removal of the thyroid gland previous to treatment.[190] However, the changes in the skeleton are less accentuated than those in animals with the thyroid gland intact.

Anterior Hypophysial Hormone and Estrogen.—In hypophysectomized rats estrogen failed to cause hyperossification.[191] In rats,[192] mice and guinea pigs [27c] with the hypophysis intact each hormone exerts its own peculiar effect on the receptor tissue. At the early stages the inhibition of growth of cartilage caused by estrogen and its stimulation by anterior hypophysial hormone antagonize each other. Therefore, an intermediate response of the cartilage results: There is less stimulation of growth than after administration of anterior hypophysial substance only and less inhibition than after treatment with estrogen alone. Likewise, increased softening and liquefaction of cartilage indicate the influence of the anterior hypophysial hormone, whereas the hyalinization so typical of the effect of estrogen is less marked. At later stages the resorption of cartilage and bone proceed at a faster rate than after treatment with estrogen alone.

According to these results, anterior hypophysial hormone does not act by direct neutralization of estrogen as has been suggested by Zondek [155c] on the basis of findings in rats and cocks treated with preparations of the two hormones. This author also contends that the growth-inhibiting action of estrogen is due to a decreased release of anterior hypophysial hormone and not to a direct effect on the growing cartilage. The histologic investigations, however, do not confirm this view, especially since the skeletal tissue changes in hypopituitarism are different from those seen after administration of estrogen.[192]

Anterior Hypophysial Hormone and Castration.—In castrated young guinea pigs [150] and rats [193] anterior hypophysial hormone likewise exerts its influence on skeletal growth and aging. In such animals skeletal aging is less accelerated than in animals with intact sex glands; on the other hand, skeletal aging is hastened as compared with conditions in the castrated animal.

Estrogen and Progesterone.—In immature mice and guinea pigs [27c] the administration of these two hormones causes a decrease of the effects exerted by estrogen

189. (*a*) Evans, H. M.; Simpson, M. E., and Pencharz, R.: Endocrinology **25**:175, 1939. (*b*) Becks, W.; Ray, R. D.; Simpson, M. E., and Evans, H. M.: Arch. Path. **34**:334, 1942. (*c*) Marx, W.; Simpson, M. E., and Evans, H. M.: Proc. Soc. Exper. Biol. & Med. **49**:594, 1942. (*d*) Laqueur E.; Dingemanse, E., and Freud, J.: Acta brev. Neerland. **11**:46, 1941. (*e*) Griffith, M., and Young, F. C.: J. Endocrinol. **3**:96, 1942. Smith.[62]

190. Richardson.[83a] Silberberg.[92a] Silberberg and Silberberg.[92b]

191. Kibrick, E. A.; Simpson, M. E.; Becks, H., and Evans, H. M.: Endocrinology **31**:93, 1942.

192. Gaarenstroom, J. H., and Levie, L. H.: J. Endocrinol. **1**:420, 1940.

193. Evans, H. M., and Simpson, M. E. Anat. Rec. **35**:36, 1927. Van Wagenen, G.: Am. J. Physiol. **84**:468, 1928. Moon, H. D.: Proc. Soc. Exper. Biol. & Med. **37**:34, 1937.

alone. The histologic appearances of the long bones resemble more closely those seen under normal conditions. Thus, progesterone apparently neutralizes at least temporarily the aging as well as the growth-inhibiting effect of estrogen. Under prolonged treatment no counteracting effect of the progesterone on the medullary bone formation caused by estrogen is observable in mice.[163e] However, in guinea pigs, in which the bone-forming effect of estrogen is not so marked, progesterone is able to counteract the effects of estrogen more successfully during the growth period. On the other hand, progesterone has a far more temporary effect than estrogen, and therefore it ceases to act, while the estrogen still continues to exert its effect on cartilage and bone.[27c]

Estrogen and Androgen.—In mice estrogen and androgen apparently interfere with each other at different times in a different way.[27c] At the early experimental stages each supports the other. The inhibition of both growth and resorption of cartilage is first more accentuated than after treatment with either hormone alone. However, soon afterward there is less bone present than after treatment with estrogen only. At these stages the testosterone may already promote resorptive processes, whereas the estrogen still inhibits resorption. During this phase, therefore, the two substances seem to counteract each other, as reported by Gardner and his co-workers.[194] Finally, again the resorption does not make corresponding progress and an interlaced trabecular network can still be found after one year's treatment. In such instances, the effect of testosterone might have ceased altogether, whereas that of estrogen still continues.[27c]

Estrogen and Thyroid Hormone.—Subsequent to the administration of these two hormones, the inhibition of growth of cartilage is less accentuated than after treatment with estrogen alone. Thyroid hormone likewise counteracts the inhibition of resorption and the new formation of bone called forth by the estrogen. Thus, rapid resorption of cartilage and complete removal of the excess bone produced by estrogen ensue. At later stages the structure of the long bones resembles that seen after administration of thyroxin alone.[27c] Thyroxin acts at least partly as an antagonist to estrogen, although both substances, if given separately, accelerate skeletal development and aging.

PROLONGED ADMINISTRATION OF HORMONES

In a general way, the described effects on skeletal growth and aging diminish with prolonged administration of hormonal preparations; they finally cease or may even be reversed.[195] This may be due to adaptation of the organism as a whole or of the skeletal tissues in particular to the hormonal stimulation. This adaptation of cartilage and bone agrees with corresponding observations of Loeb and associates[196] with respect to the thyroid gland and ovaries. In all these tissues gradual adaptation occurs, irrespective of the chemical nature of the hormones, whether they are proteins, as are, for instance, the anterior hypophysial factors, or cyclic hydrocarbons as are some estrogens and androgens. This rule applies even to the simple inorganic salt potassium iodide. The least degree of adaptation is seen in the case of thyroid hormone.

194. Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **38**:599, 1938. Gardner.[164b]

195. Silberberg and Silberberg.[27c] Mortimer.[48a] Johnson and Hill.[67d] Downs.[70a]

196. Loeb, L., and Friedman, H.: Proc. Soc. Exper. Biol. & Med. **29**:172, 1931. Paul, M.; Schmeckebier, M. M., and Loeb, L.: Endocrinology **19**:329, 1935.

SIGNIFICANCE OF THE AGE FACTOR

The older the animals are at the beginning of the treatment, the greater is the resistance they oppose to the action of the hormones. Young adult animals react less readily than growing animals, and old animals are even more refractory.[197]

The dose has to be considerably increased in order to elicit a response. Similarly, strains exhibiting a slow rate of skeletal growth and aging respond more readily than strains showing rapid skeletal growth and aging.[27e] This may be due to the fact that at any given chronologic age the skeletal age of a slowly aging strain is less advanced than that of a fast aging strain.

In young adult and old animals that have ceased to grow at the beginning of the treatment neither anterior hypophysial preparations nor thyroid hormone nor potassium iodide is able to revive the proliferation of the epiphysial cartilage. However, the aging processes that are in progress at the initiation of the experiment can be accelerated and intensified. Moreover, the incidence and the severity of arthropathic lesions can be increased and their onset hastened under the influence of anterior hypophysial substance.[198]

Administration of estrogen or androgen increases the resorption of the shafts of the bones in old animals [199]; this is followed by new formation of bone. As in the growing skeleton, the effects of testosterone are generally milder.

IS THE ACTION OF A HORMONE ON THE SKELETON SPECIFIC?

As has been discussed, the skeletal effect of any particular hormone is not the same under all conditions; it varies with the species and the strain, the age and the sex of the animal to which the hormonal preparation is given; likewise, seasonal influences,[200] the dose administered, the mode of application [201] and the duration of the experiment may modify its action.

Moreover, the administration or the withdrawal of the hormone of one organ may affect the function of other endocrine organs. Therefore, certain changes in cartilage and bone may be attributable to the hormone itself or to the intermediation of other glands. The interpretation of the skeletal changes becomes even more difficult because of the peculiar response of these tissues to alterations of their normal structure. Increased bone formation of whatever origin is followed by intensified resorption and, vice versa, increased resorption is followed by intensified formation of bone. This reaction is probably due to local factors acting on the mesenchymal tissues. Similarly, in the joints increased regression of cartilage and bone stimulates growth processes, whereas increased new formation of cartilage and bone may be followed by increased regression. These local conditions may play a role in the reversal of the hormonal effects seen particularly under prolonged administration. Thus the question arises: Is the reversal due to the hormonal action, or does it take place in spite of the hormonal stimulation, or does it occur because of the waning effect of the hormone?

In view of these circumstances the question as to the specificity of the hormonal effects on the skeleton becomes most perplexing. Thus, the skeletal effect of estrogen is not simple and uniform; it causes inhibition as well as increase of resorption of bone. Furthermore, comparable skeletal changes have been produced

197. Silberberg, M., and Silberberg, R.: Anat. Rec. **78**:549, 1940. Jaffe and others.[118b] Silberberg and Silberberg.[27e] Handelsman and Gordon.[67f] Binswanger.[89e]

198. Silberberg and Silberberg.[27e] Silberberg and Silberberg.[70g]

199. Silberberg, M., and Silberberg, R.: Anat. Rec. **80**:347, 1941.

200. Gardner.[164b] McLean.[164c]

201. Silberberg and Silberberg.[154b] Zondek.[155d]

by the administration of molecular phosphorus,[202] and of vitamin D[203] and in vitamin A deficiency.[203] These and similar observations indicate that the action of estrogen on the skeleton may be of a more general nature.

More recently a number of cases have been made known in which fibrous osteitis was associated not only with hyperparathyroidism but also with hyperthyroidism and precocious skeletal and sexual maturity.[204] Such cases demonstrate again how difficult it may be to determine the primary disturbance and to prove the specificity of its effect on the skeleton.

Similarly, the data regarding the specificity of the so-called growth hormone of the anterior lobe of the hypophysis are not entirely satisfactory for three reasons: First, the presence of an eosinophilic adenoma in many cases of acromegaly does not prove that the skeletal hypertrophy seen in this disease is produced by a single specific growth hormone. Skeletal changes resembling those of acromegaly may occur without eosinophilic adenoma and even without any involvement of the anterior lobe of the hypophysis.[205] It has not been convincingly shown that in such cases there is exhaustion atrophy of the eosinophils after preceding hyperplasia.[6c] On the other hand, eosinophilic adenoma of the anterior lobe of the hypophysis may be found without hypertrophic skeletal changes.[206] Finally, acromegaly is frequently associated with sexual dysfunction, with pathologic lactation, even in the male, and with hyperplasia of the thyroid and parathyroid glands,[207] conditions that may themselves influence skeletal growth and aging. Therefore, in acromegaly a number of hormones may be responsible for the skeletal changes, and while there is some specificity in the hormonal action, this specificity is restricted and quantitative rather than absolute. What holds good in the case of acromegaly may equally apply in various other abnormal processes affecting the skeletal system.

Second, with regard to the arrest of growth subsequent to removal of the hypophysis, body growth may go on for some time after hypophysectomy; nor can the effectiveness of the administration of a preparation containing the so-called growth hormone to the hypophysectomized animals be considered as decisive, because this replacement does not explain the nature of the hormone; even under these conditions the restoration of growth is only incomplete.

Third, nonpituitary substances, such as thyroxin, testosterone, chorionic gonadotropin and potassium iodide, may under certain conditions enhance bone growth.

Ultimately, skeletal growth is the result of a variety of complex biochemical processes. It does not seem probable that a single growth hormone could promote all of them and still, even in a limited way, be specific in its function. This restriction is particularly indicated by the fact that some pituitary as well as nonpituitary

202. Adams, C. O., and Sarnat, B. C.: Arch. Path. **30**:1192, 1940. Gall, E. A.; Barr, J. S., and Lingley, J. R.: Proc. Soc. Exper. Biol. & Med. **45**:539, 1940.

203. Eddy, W. H., and Dalldorf, G.: The Avitaminoses, ed. 2, Baltimore, Williams & Wilkins Company, 1941.

204. Pehn, N.; Policard, A., and Dufourt, A.: Presse méd. **39**:999, 1931. Albright, F.; Butler, A. M.; Hampton, A. O., and Smith, P.: New England J. Med. **216**:727, 1937. McCune, D. J., and Bruch, H.: Am. J. Dis. Child. **54**:806, 1937. Sternberg, W. H., and Joseph, V.: ibid. **63**:748, 1942. Summerfeldt, P., and Brown, A.: ibid. **57**:90, 1939. Lichtenstein, L., and Jaffe, H. L.: Arch. Path. **33**:777, 1942.

205. Bleibtreu, L.: Münchem. med. Wchnschr. **52**:2079, 1905. Petrén, K.: Virchows Arch. f. path. Anat. **190**:1, 1907. Moskalew, M.: ibid. **201**:289, 1911. Bailey and Cushing.[6c]

206. Cuervo, L. V.: Vida nueva **43**:123, 1939. Bailey and Cushing.[6c]

207. Moehlig, R. C.: Endocrinology **25**:134, 1939. Davis, A. C.: J. Clin. Endocrinol. **1**:445, 1941. Staemmler, M.: Klin. Wchnschr. **19**:1231, 1940. Stephens, D. J.: Endocrinology **25**:638, 1939.

substances affect the protein metabolism, the intake of water and the mineral metabolism, especially that of calcium and phosphorus, and the phosphatase activity.[208]

In the growing organism these functions may well be utilized for skeletal growth; disturbances of these functions necessarily lead to impairment of growth owing to the ensuing deficiency of materials essential for body growth.

On the other hand, anterior hypophysial as well as other hormones promote not only growth but also regression and resorption of cartilage and bone; in other words, the same substances that are capable of stimulating growth promote also the opposite condition, namely, the regressive changes of aging. Indeed, the latter effect seems to outbalance in some instances the growth-promoting functions of these hormones.

Presumably, a number of hormones cooperate also in various metabolic activities in order to bring about skeletal aging. However, knowledge of the biochemical processes leading to aging of the skeleton is still too limited to permit at present any conclusions as to the mechanisms involved.

SUMMARY

The course of skeletal growth and aging is primarily genetically determined. However, in the normal organism several hormones participate in regulating the relations of skeletal growth and aging. Endocrine disorders may affect this relationship. They may accelerate and intensify or delay and diminish one or all phases of the skeletal time curve. Frequently these effects are temporary only and are compensated at later stages. However, depression of growth and acceleration of aging may be so markedly intensified that permanent stunting results. But it has not yet been proved that addition or withdrawal of hormones stimulates skeletal growth and aging beyond the limits set by the genetic constitution of the organism.

208. Williams, H. L., and Watson, E. M.: Endocrinology **29**:250 and 258, 1941. Whicher, C. H., and Watson, E. M.: ibid. **33**:83, 1943.

Notes and News

Appointments and Retirements.—In Western Reserve University, Cleveland, Simon Koletsky has been promoted to an assistant professorship in pathology.

Dorothy Loomis has been appointed assistant professor of pathology in Long Island College of Medicine, Brooklyn, N. Y.

Cornelius P. Rhoads, director of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, has been granted leave of absence to become chief of the Medical Division of the Chemical Warfare Service of the United States Army with the rank of colonel.

Jay McLean, of the Memorial Hospital, New York, has been appointed associate professor of surgical research at the Ohio State University.

Burton Simpson, director of the State Institute for the Study of Malignant Disease, Buffalo, since 1924, retired last August 1.

William Hibbs, pathologist at the Children's Memorial Hospital, has accepted appointment as medical director of the Presbyterian Hospital, Chicago.

Deaths.—Roscoe Raymond Hyde, professor of immunology and director of the laboratories of immunology and filtrable viruses of the Johns Hopkins University School of Hygiene and Public Health, died on September 15 at the age of 59 years.

Louis B. Wilson, for many years at the head of the section on pathology at the Mayo Clinic, then director of the Mayo Foundation, died October 5, aged 76. He was a leader in graduate medical education and served on the Council on Medical Education and Hospitals, also on the National Board of Medical Examiners.

Antonio Fontes, director of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil, and well known for his contributions to the biology of the tubercle bacillus and of plague, has died at the age of 64. Not long ago his name was inscribed in the Brazilian Book of Merit.

Finney-Howell Research Foundation, Inc.—All applications for fellowships for next year must be filed in the office of the Foundation, 1211 Cathedral Street, Baltimore, by Jan. 1, 1944. Applications received after that date cannot be considered for 1944 awards, which will be made March 1, 1944.

This foundation was provided for in the will of the late Dr. George Walker, of Baltimore, for the support of "research work into the cause or causes and the treatment of cancer." The will directed that the surplus income from the assets of the foundation together with the principal sum should be expended within a period of ten years to support a number of fellowships in cancer research, each with an annual stipend of $2,000, "in such universities, laboratories and other institutions, wherever situated, as may be approved by the Board of Directors."

Fellowships carrying an annual stipend of $2,000 are awarded for the period of one year, with the possibility of renewal up to three years; when deemed wise by the Board of Directors, special grants of limited sums may be made to support the work carried on under a fellowship.

Applications must be made on the blank form which will be furnished by the Secretary or any member of the Board of Directors.

The D. J. Davis Lectureship on Medical History.—This lectureship has been established by friends and colleagues of D. J. Davis on his retirement as dean and professor of pathology at the University of Illinois College of Medicine.

Cancer Research at University of Minnesota.—According to current reports the legislature has made an annual appropriation of $15,000 to the medical school for special research in cancer; the Citizens Aid Society will continue annual grants of $5,500 for the George Chase Christian professorship in cancer research and of $10,000 in support of cancer education and research; the Jane Coffin Childs Memorial Fund has granted $5,000 for work on the milk factor in mammary cancer of mice.

Typhus Commission.—By executive order the United States of America Typhus Commission has been established in the War Department "for the purposes of protecting the members of the armed forces from typhus fever and preventing its introduction into the United States." Col. S. Bayne-Jones has been appointed director of the commission.

Obituaries

LESLIE TILLOTSON WEBSTER, M.D.
1894–1943

Dr. Leslie Tillotson Webster died July 12, 1943, in his forty-ninth year, when his work was expanding into new fields, for his restive intellect was in continual activity and his achievements reached increasingly higher levels. He wrote his own eulogy.

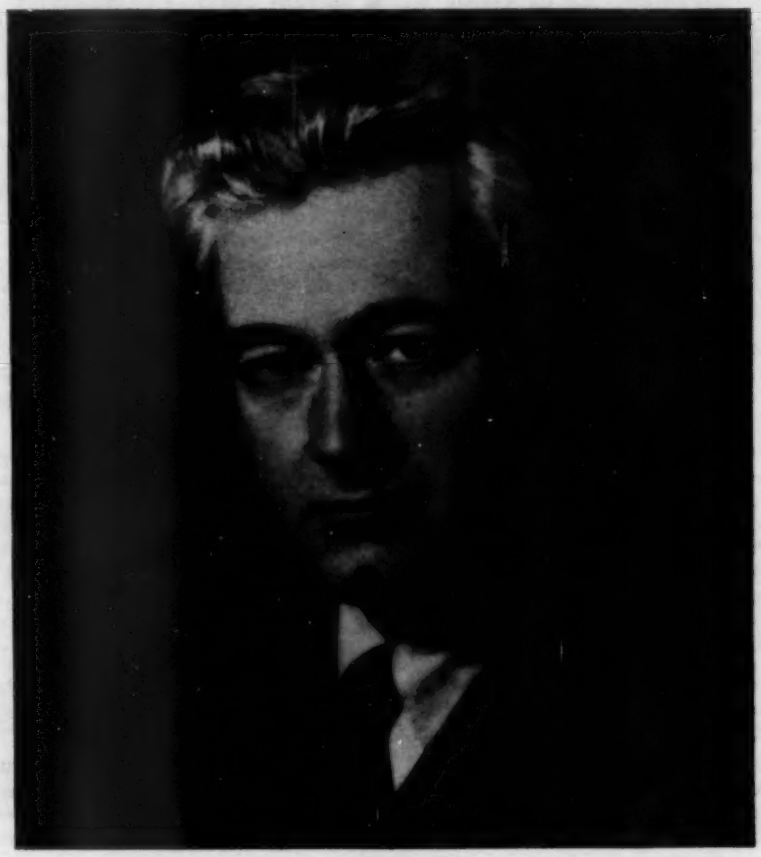
He was born in New York, July 23, 1894. Amherst College gave him the degree of Bachelor of Arts in 1915 and twenty years later decorated him with an honorary Doctorate of Science. His medical degree was won at the Johns Hopkins University in 1919, and after a year in the department of pathology there he came to the Rockefeller Institute for Medical Research, where he rose in rank to membership (1934). In 1924 he married Emily Johnston de Forest II—faithful, valiant companion, devoted to him and his work; both fought his illness with admirable courage.

His first work was in experimental epidemiology. He was to become the foremost investigator in this subject in America, developing new concepts. One fact discovered by him was that individual members of a given population subject to epidemics varied in their susceptibilities to the specific infection and that this host factor, as well as the dosage of the infective agent, controlled the waxing and waning of epidemics—rather than the inherent virulence of the prevailing infective agent, for in his experiments he discovered that virulence remained stable. What is equally important is that he evolved a technic of experimental epidemiology which was quantitative and yet reproduced the natural phenomena of infection and its spread in a population. His chief results were presented in a Harvey Lecture (1932) in which he showed that when a foreign micro-organism entered a hitherto unexposed community the extent and severity of the infection were determined by the inherent virulence, the available dosage and the amount and distribution of nonspecific resistance in the population. If the micro-organisms were already present, variations in the resistance of the population and in the available dosage were chiefly responsible for the degree of spread. Fluctuations in the resistance of the population were induced by immigration, season and diet acting on the nonspecific components and by the host's developing the specific immune response. Fluctuations in the dosage arose from variations in host resistance and vector factors. The variable resistance displayed by individuals he found to be inheritable, and by inbreeding he developed strains of mice which were highly susceptible to specific infection by viruses or by bacteria and strains highly resistant to such infections. At the time of his death he was chairman of the National Research Council Committee on the Maintenance of Pure Genetic Strains and had initiated an energetic and comprehensive inquiry (with his associate Dr. H. A. Schneider) into the influence of dietary factors on resistance and susceptibility to infections.

Dr. Webster was one of the pioneers in the use of the albino mouse for the systematic study of problems of infection. He advanced the study of experimental virus infections immeasurably by showing, in 1933, with Dr. G. W. Fite, that

suspensions (louping ill virus) simply dropped onto the nasal membranes, without injuring them, would induce the characteristic disease.

In recent years, up to the time of his death, he was engaged in studies on encephalitic viruses and such diseases as poliomyelitis, rabies, encephalitis of the St. Louis, Japanese B and Russian types, equine encephalomyelitis and West Nile fever. In his investigations on rabies he devised a more exact procedure for diagnosis by means of mouse inoculation, also a mouse test for measuring the potency of vaccine. With Dr. Casals he developed an antirabic vaccine of the virus killed by ultraviolet radiation, which he proved effective even in a single dose in



LESLIE TILLOTSON WEBSTER, M.D.
1894–1943

experimental animals. He also wrote a book for the general reader, "the first comprehensive, modern scientific study ever published," entitled "Rabies" which should be of importance in controlling the disease. Owing to his interest and contributions in the field of experimental and human infectious encephalitis, he was invited to collaborate with the Neurotropic Virus Commission of the United States Army.

Pathologists owe him—*non omnis moriar*—a deep debt of gratitude for his achievements which will "animate eternal years."

PETER K. OLITSKY.

Books Received

THE BIOCHEMISTRY OF MALIGNANT TUMORS. Kurt Stern, M.D., formerly research associate of the University of Vienna, and Robert Willheim, M.D., professor, University of Philippines, Manila. Pp. 951. Price $12. Brooklyn: Reference Press, 1943.

This book continues the review of chemical cancer research by the same authors published seven years ago (Willheim, R., and Stern, K.: Die Wege und Ergebnisse chemischer Krebsforschung, Vienna, Aesculap-Verlag, 1936, pp. 473). Circumstances have placed the greater share of the responsibility for the present volume on Kurt Stern. The literature to the end of 1941 is covered and in part also that of 1942. A new chapter has been added on the biochemical aspects of origin and growth of tumors; otherwise the plan is that of the first issue. The other topics are: inorganic chemistry, organic chemistry, physical chemistry, enzymes, nutrition and vitamins, metabolism, endocrine glands and hormones, immunology, chemical and biologic tumor diagnosis. The text is mostly free from authors' names, the references being conveniently placed at the bottom of the pages and numbered consecutively for each chapter. For example, the number of references for the chapter on organic chemistry in relation to cancer is 592 in the first issue and 1,011 in the second, the authors' index of which includes no less than some 3,500 names. The subject index is adequate. The book seems to be remarkably free from errors and misprints. The style is clear and fluent, at times verging on redundancy. The book gives a comprehensive and well organized survey of the results of biochemical cancer research. It will be of service to all who are interested in the advances in this field.

FRONTIERS IN CYTOCHEMISTRY: THE PHYSICAL AND CHEMICAL ORGANIZATION OF THE CYTOPLASM. Edited by Normand L. Hoerr, Henry Wilson Payne professor of anatomy, Western Reserve University School of Medicine. Biological Symposia, volume X. Pp. 334, illustrated. Price $3.50. Lancaster, Pa.: The Jaques Cattell Press, 1943.

Last November a symposium on the chemical and physical organization of cytoplasm was held in honor of the seventy-fifth birthday of R. R. Bensley, professor emeritus of anatomy in the University of Chicago. About 500 people attended the symposium. The event created so much interest that the contributions have been published in the present volume. Besides the editor's foreword, which is a survey of Dr. Bensley's scientific work and an appreciation of Dr. Bensley by E. V. Cowdry, this interesting and well edited book contains fourteen articles by scientists engaged in active investigation of the physical and chemical problems of cytoplasm. It contains a good portrait of Dr. Bensley and ends with his paper on the chemical structure of cytoplasm.

SYNOPSIS OF TROPICAL MEDICINE. Sir Philip Manson-Bahr, C.M.G., D.S.O., M.D., F.R.C.P., senior physician to the Hospital for Tropical Diseases, etc. Pp. 224, with 5 plates. Price $2.50. Baltimore: Williams & Wilkins Company, 1943.

Manson-Bahr's synopsis includes all the diseases of the tropics, infectious, nutritional, climatic and metazoal, also the vegetable and animal poisons. It is published in response to the need for a compact and up-to-date guide on the part of medical military officers and others called by the exigencies of war to the tropics. It does not aim to replace standard textbooks in tropical medicine.

CORRECTIONS

In the August issue (ARCH. PATH. **36**:233, 1943) a statement was published to the effect that Dr. Burrell O. Raulston has been appointed professor of bacteriology at the University of Southern California, Los Angeles. This statement was in error.

In the article by Dr. Franz Wenger entitled "Incomplete Rupture of the Aorta, Not Followed by Dissecting Aneurysm: Report of Two Cases" in the September issue (ARCH. PATH. **36**:253, 1943), the phrase "(as was the general view among the earlier pathologists)" in the twenty-seventh line on page 260 should be placed after the word "rupture" near the end of the first line in the paragraph.